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THE STABILITY OF IODINE IN IODIZED ROCK SALT[1]

W. M. DAVIDSON[2] AND C. J. WATSON[3]

Division of Chemistry, Central Experimental Farm, Ottawa, Canada

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As insurance against goitre in livestock, iodine has been incorporated into stock salt. The efficiency of this agent will be influenced by its stability under practical farm conditions. Studies on iodized salt for human consumption have revealed losses of iodine. For instance, Andrew (1) made a survey of the iodine content of salt in 1½ lb. cartons and 5 lb. bags. He found that the iodine was lower in the samples stored in cartons than in those stored in bags. On the basis of his experiments the author concluded that the losses were due chiefly to absorption by the container. The addition of magnesium carbonate did not inhibit the loss of iodine.

Proposals have been made for the addition of chemical agents to prevent these losses. For instance, at the Mellon Institute, Johnson and Frederick (3) suggested the use of calcium stearate. To 92 parts of potassium iodide, 8 or more parts of calcium stearate were added in the form of an impalpable powder. They found that iodized mineral feed mixtures lost between 9 to 20 per cent of their iodine during four months of storage under ordinary conditions.

Chilcorn, Greim and Hart (2), at Wisconsin, proposed the use of sodium thiosulphate. To each ton of salt were added 2 lb. of sodium thiosulphate, 2 lb. of sodium carbonate, 2 lb. of starch and 0.4 lb. of potassium iodide.

The present paper reports an investigation in which the losses of iodine from iodized salt, with and without protective agents and with and without the addition of ferric oxide, were determined under practical feeding conditions. This was carried out in co-operation with Merck & Co. Limited, Montreal, Quebec, and the Goderich Salt Company Limited, Goderich, Ontario.

Merck & Co. prepared the salt mixtures. The Goderich Salt Company prepared the pressed blocks and also supplied loose salt, corresponding in composition to the pressed blocks. The salt mixtures prepared were as follows:

Number One: 1 oz. of potassium iodide made up to 5 lb. with sodium chloride.

Number Two: 1 oz. of potassium iodide
10 oz. of iron oxide red No. 1068 made up to 5 lb. with sodium chloride.

Number Three: 1 oz. of potassium iodide
5 oz. soda thiosulphate anhydrous
5 oz. soda carbonate anhydrous
5 oz. corn stach and made up to
5 lb. with sodium chloride.

[1] Contribution No. 138 from the Division of Chemistry, Science Service, Dominion Department of Agriculture, Ottawa.
[2] Agricultural Assistant, Science Service.
[3] Chemist, Science Service.

Number Four: 1 oz. of potassium iodide
5 oz. of soda thiosulphate anhydrous
5 oz. of soda carbonate anhydrous
5 oz. of corn starch
10 oz. iron oxide red No. 1068 and made up to
5 lb. with sodium chloride.

Number Five: 1.1 oz. potassium iodide and calcium stearate mixture and made up to 5 lb. with sodium chloride.

Number Six: 1.1 oz. potassium iodide and calcium stearate mixture
10 oz. iron oxide red No. 1068 and made up to
5 lb. with sodium chloride.

Number Seven: Control sample of 5 lb. of sodium chloride.

From these mixes the Goderich Salt Company made up a 300 lb. batch of salt. From this they pressed a 50 lb. block of each mix. In addition they supplied 10 lb. of loose salt, representative of the material from each block.

There were, therefore, seven varieties of block salt. These were subjected to three different treatments. These will be referred to as pasture conditions, stall conditions, and storage conditions. The granulated samples were stored in glass jars.

Under pasture conditions two 5 lb. blocks of rock salt were sawn from the original 50 lb. block. These were placed on a shelf in the open and subjected to wind, rain and sun at all times. Under stall conditions, two more 5 lb. blocks were sawn off and placed on wooden slabs in the nutrition barn, exposed to the air. They were sprinkled each day with water and scrubbed for a few minutes. Under storage conditions the remainder of each block of rock salt was placed in cardboard cartons and stored in a well ventilated dry shelf in the nutrition barn. For storage in bottles the loose salt was placed in half-gallon sealers.

The original iodine content of the blocks was determined in May, 1942, by sawing a small sample from each of the 50 lb. blocks. The iodine content of the salt under pasture conditions was determined in July, 1942. For this purpose the entire 5 lb. block was ground up and the iodine extracted. Under stall conditions, determinations were made in July 1942, February, 1943, and September, 1943. Under storage conditions determinations were made in August, 1942, and February, 1943. In both cases 100 g. samples were sawn off from each block for analysis.

TABLE 1.—IODINE CONTENT OF IODIZED ROCK SALT UNDER PASTURE CONDITIONS

Mix No.	Description	Iodine content in parts per 100,000	
		Original May 1942	July 1942
1	NaCl + Kl	11	0
2	NaCl + Kl + Fe_2O_3	11	0
3	NaCl + Kl + $Na_2S_2O_3$	13	Traces
4	NaCl + Kl + $Na_2S_2O_3$ + Fe_2O_3	14	0
5	NaCl + Kl + Ca Stearate	11	0
6	NaCl + Kl + Ca Stearate + Fe_2O_3	13	0
7	NcCl	4	0

TABLE 2.—IODINE CONTENT OF IODIZED ROCK SALT UNDER STALL CONDITIONS

Mix No.	Iodine content in parts per 100,000			
	Original May 1942	July 1942	February 1943	September 1943
1	11	4	2	1
2	11	10	3	2
3	13	4	4	Traces
4	14	13	3	2
5	11	7	9	Traces
6	15	6	5	2
7	4	1	Nil	Nil

For the loose salt stored in glass jars, original determinations were made in May, 1942, and subsequent determinations in August, 1942, and June, 1943.

The method of analysis was based on the work of von Fellenberg (4).

METHOD OF ANALYSIS OF SALT

The reagents used were:

(*a*) *Water*—redistilled from alkaline carbonate.
(*b*) *Ethyl alcohol*—95 per cent redistilled from alkaline carbonate.
(*c*) *Carbon tetrachloride*—redistilled from alkaline carbonate.
(*d*) *Potassium carbonate*—washed with redistilled alcohol and made up into a saturated solution with redistilled water.
(*e*) *Potassium nitrate*—5 per cent solution made up with redistilled water.
(*f*) *Dilute sulphuric acid*—5 per cent solution made up with redistilled water.

In the case of the block salt, the sample was ground in a mortar; 100 g. of ground salt were made into a paste with potassium carbonate solution. This was extracted three times with boiling alcohol, filtered and washed with alcohol. The combined filtrates were evaporated to dryness. The residue was dissolved in water, transferred to a separatory funnel and the iodine liberated by the addition of 5 ml. of potassium nitrite and 5 ml. of dilute sulphuric acid. The iodine was extracted with carbon tetrachloride, made up to volume in a volumetric flask and the intensity of colour determined in a Klett-Summerson colorimeter.

The possibility that some iodate might be formed was tested in the case of the block salt under pasture conditions. 30 g. samples were dissolved in 250 ml. water. A fraction of a gram of sodium bisulphite was added and the solution acidified with hydrochloric acid. It was boiled, made alkaline with potassium carbonate and evaporated to a paste. The above procedure was then followed. No iodine was found.

Iodine standards were prepared from potassium biniodate. This was reduced with sulphurous acid and the iodine finally extracted with carbon tetrachloride following the above procedure.

The results are given in Tables 1 to 4.

In the summer pasture conditions there was a rapid loss from all samples. Under stall conditions the losses were less severe than under pasture conditions. After two months there was still considerable iodine left. There were small amounts after eleven months. Finally, after 17

TABLE 3.—IODINE CONTENT OF IODIZED ROCK SALT STORED IN CARDBOARD CARTONS

Mix No.	Iodine content in parts per 100,000		
	Original May 1942	August 1942	February 1943
1	11	9	12
2	11	9	21
3	13	20	24
4	14	18	24
5	11	11	13
6	13	18	6
7	4	3	5

TABLE 4.—IODINE CONTENT OF GRANULATED IODIZED SALT STORED IN GLASS JARS

Mix No.	Iodine content in parts per 100,000		
	Original May 1942	August 1942	June 1943
1	18	16	15
2	13	12	11
3	17	14	11
4	17	19	16
5	16	10	12
6	13	14	17
7	9	5	5

months most of the iodine had disappeared. While there was no noticeable effect due to preservatives, samples 3 to 6 containing the preservatives tended to have slightly higher values of iodine at the end of 11 months.

In the case of samples stored in cartons, there was no general loss of iodine. In certain cases the values at the end of the storage period were higher than those originally. This may have been partly due to some lack of uniformity in distribution and partly to the possible movement of the iodine towards the surfaces of the blocks.

As shown in Table 4, there were no losses of the loose salt stored in bottles. These data did not show the increases encountered in blocks stored in cartons. Being loose, the sampling was more efficient and there would be no migration of iodine.

A further test under pasture conditions was made. A block of salt containing potassium iodide and sodium thiosulphate (Mix *No. 4*) was placed outside in October to be weathered under winter conditions. In April it still contained 11 parts per 100,000 of salt. The winter was generally severe.

As a final experiment a series of small cubes were exposed to pasture conditions commencing in April, 1943, and examined weekly. The results are shown in Table 5. As will be seen the iodine had practically disappeared at the end of six weeks.

TABLE 5.—IODINE CONTENT OF CUBES OF IODIZED SALT UNDER SUMMER PASTURE CONDITIONS. MIX NO. 4

Length of exposure	Iodine content in parts per 100,000
0 weeks	14
1 week	8
2 weeks	6
3 weeks	3
4 weeks	4
5 weeks	6
6 weeks	2

SUMMARY AND CONCLUSIONS

1. Iodized rock salt under summer pasture conditions rapidly loses its iodine.

2. Under stall conditions, losses are less rapid and the iodine shows fair stability up to two months. After seventeen months only traces or small amounts of iodine were present.

3. Under good storage conditions in cardboard cartons there was no loss of iodine over a nine-month period.

4. Iodine in loose salt stored in glass jars remained stable for periods up to fifteen months.

5. It is recommended that iodized rock salt for stall feeding should be fed in such quantity that it is consumed in less than two months. Under those conditions it will retain a large percentage of its iodine.

6. Protective agents may tend to reduce losses of iodine but this fact could not be definitely demonstrated from the data.

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INTERRELATIONS OF COMMON ROOT ROT AND OTHER FACTORS WITH WHEAT YIELDS IN SASKATCHEWAN[1]

B. J. SALLANS[2]

Dominion Laboratory of Plant Pathology, Saskatoon, Sask.

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INTRODUCTION

The losses in wheat yields caused by common root rot (*Helminthosporium sativum* P. K. and B. and *Fusarium* spp.) in Western Canada have been estimated by various means in the past several years. Craigie (3) reported on the basis of experimental plot results at Winnipeg that average annual losses in Manitoba were not less than 5 per cent of the crop and in some years were much more. From studies by the writer (13), it appears that subcrown internode lesions classed as "severe" reduced individual plant yields by 30 to 45 per cent as compared to the yields of neighbouring healthy or slightly lesioned plants. Similarly, moderate lesions reduced yields by 15 to 20 per cent. Severe lesions were present in 10 to 35 per cent and moderate lesions in 12 to 30 per cent of the plants taken from a number of wheat fields. Machacek (9) used somewhat similar data to estimate yield reductions in Manitoba. Over the three year period 1939-41, he estimated losses of 8.0, 16.4, and 12.1 per cent in the successive years. Machacek refers also to unpublished estimates assembled by F. J. Greaney of losses of 5, 9.4, and 3 per cent for Manitoba, Saskatchewan, and Alberta, respectively.

The difficulties in the way of making accurate estimates of losses are numerous and diverse. Common root rot is present in all parts of Saskatchewan year after year, the organisms overwintering chiefly on crop refuse on or near the surface of the soil. The disease does not ordinarily occur in distinctly delineated areas in the fields but is present in plants more or less scattered throughout the crop. The plants grow in competition largely with others which may be healthy or more or less diseased. The incidence of the disease varies greatly from early till late in the season. Plants for the most part are attacked after the seedling stage, commencing usually in early June. The great increase in the number of plants attacked may be in the last two weeks of June one year or the first two weeks of July in another year. This may make considerable difference in the effects of the disease on final yields, since it appears unlikely that late infections will cause as much injury as the earlier ones. There is also the influence of recovery of the plant from early infections to be considered. Plants which escape pre-emergence and seedling blight, phases of the disease which are seldom important in Saskatchewan, usually survive attacks by *Helminthosporium sativum*. At first, such plants may be severely stunted in growth in consequence of the disease, but they appear to throw off the effects of the fungus and, under favourable conditions of moisture, temperature, light, and competition, may regain or even more than regain the growth initially lost (14). Weeds are also a complicating factor since if wheat plants are stunted by root rot, weeds are better able to develop and may in part prevent any large degree of recovery of the crop from a poor start (15).

[1] Contribution No. 918 from the Division of Botany and Plant Pathology, Science Service, Dominion Department of Agriculture, Ottawa, Canada.

[2] Associate Plant Pathologist.

Other factors, such as drought, enter into the picture. It is very difficult to determine how much injury is ascribable to drought and how much to root rot.

It is the object of the author in this paper to present an analysis of certain data collected by way of surveys over the past ten years in Saskatchewan in the hope that it may serve, in a preliminary way at least, to indicate some of the relationships between various factors, including common root rot, and yields.

DATA AVAILABLE FOR THE STUDY

Beginning in 1934, the plant disease survey in Saskatchewan was modified in so far as common root rot records are concerned so that a reasonably accurate figure or rating could be given to each field entered. This consisted in collecting an adequate number of plants at random and classifying them on the basis of subcrown internode lesions and by the use of a formula given by Russell and Sallans (12) arriving at a disease rating. To avoid influencing the disease ratings due to the observer's estimate of the crop yield, samples collected in the field were numbered, the heads and straw removed, and the ratings made at a later date in the laboratory.

The crop districts of Saskatchewan as used by the Provincial Department of Agriculture in reporting crop statistics proved very useful as units of area for the study. In the first place, each of the districts, nine in number, is more uniform as to climate and soil than is the province as a whole. In the second place, data are available on a crop district basis regarding rainfall, air temperatures, crop yields, and acreages in the annual reports of the Department of Agriculture (10, 11). In the correlation studies which follow, the root-rot ratings for individual fields in a crop district for any one year were averaged. It has not been possible, for various reasons, to use all the available data for each crop district for every year. In 1935, a very severe rust epidemic almost entirely ruined the wheat of crop districts 1, 2, and 5. In 1937, due to extreme drought conditions, the plant disease survey was not carried into certain crop districts. In various years, an insufficient number of root-rot readings were taken in various crop districts to give a reliable measure of the disease. All such units were discarded, so that out of a possible 90 (9 districts for 10 years) only 64 were available for study. These were further reduced to 59 for regression studies as insect losses were not available in 1937.

The relations of soil moisture to the prevalence of common root rot and its influence on wheat yields could only be studied from precipitation records, since soil moisture data are not available on a sufficiently extensive basis. Similarly, air temperature data were used in place of soil temperatures.

Table 1 gives wheat yields in bushels per acre by crop districts; rainfall in inches for two periods, June and July of the crop season and preseasonal rainfall, defined below; air temperatures in degrees Fahrenheit, being the averages of the daily means for June and July of the crop year; insect damage by crop districts in percentage of the crop, as reported in the annual reports of the Provincial Secretary of Statistics (10, 11) and common root-rot ratings. The common root-rot ratings are based on samples taken at usually twenty, never less than ten, places at more or less regular intervals in traversing each crop district.

TABLE 1.—WHEAT YIELDS, RAINFALL, AIR TEMPERATURES, COMMON ROOT-ROT RATINGS, AND INSECT DAMAGE BY CROP DISTRICTS IN SASKATCHEWAN

Crop district	Year	Yield	Rainfall		Temperature June-July	Common root rot	Insect damage
			June-July	Pre-season			
		bu. per acre	inches	inches	° F.	rating	per cent
1	1934	4.8	3.22	5.84	62	14.4	16.5
	1936	5.7	3.60	9.65	66	8.4	7.25
	1937	3.9	3.37	5.28	67	10.6	*
	1938	8.2	5.18	7.88	64	8.9	56.9
	1941	22.1	5.66	11.89	65	11.7	10.9
	1942	21.4	6.57	9.51	60	6.1	6.3
	1943	23.3	5.06	14.52	62	7.1	7.75
2	1934	4.8	4.90	6.89	62	14.4	26.7
	1936	9.1	3.70	9.55	67	12.0	9.2
	1937	1.5	2.68	4.79	68	10.7	*
	1938	10.1	3.82	7.50	65	8.7	47.5
	1940	13.2	5.04	5.52	63	6.8	19.7
	1941	17.8	5.46	10.27	65	10.8	17.0
	1942	23.7	7.88	9.11	60	5.4	5.45
	1943	17.8	4.42	9.73	62	10.3	8.95
3	1934	3.6	4.44	6.82	63	13.7	32.1
	1935	11.6	5.92	6.47	64	6.6	20.0
	1936	4.4	2.74	7.58	67	17.8	18.0
	1937	0.2	3.13	4.14	68	10.2	*
	1938	7.0	3.82	8.09	65	10.6	43.7
	1939	18.1	7.39	8.29	61	7.5	20.1
	1940	16.2	5.97	6.18	64	7.2	18.3
	1941	8.0	4.14	7.72	67	10.8	25.95
	1942	25.6	7.10	8.98	61	7.7	12.45
	1943	14.4	3.52	9.40	62	10.8	25.50
4	1934	4.3	4.37	6.00	62	15.2	22.3
	1937	0.1	2.84	3.84	67	13.6	*
	1938	9.9	3.40	7.64	66	11.4	16.4
	1939	17.9	6.73	8.32	61	8.0	12.2
	1940	19.4	4.89	7.23	64	6.3	15.65
	1941	11.3	5.09	8.59	66	12.1	23.5
	1943	5.7	2.98	7.42	63	8.5	39.85
5	1934	18.1	5.45	9.15	59	9.9	3.9
	1936	16.5	5.30	9.11	64	8.6	3.8
	1937	7.5	2.76	7.27	66	10.4	*
	1938	15.2	5.92	7.84	62	9.7	8.2
	1939	23.3	6.72	9.05	60	6.6	3.8
	1940	16.0	6.78	6.29	61	7.0	3.05
	1941	15.7	3.26	10.78	65	9.8	6.7
	1942	24.6	7.57	7.81	59	6.0	2.15
	1943	24.1	3.40	9.28	60	9.9	2.55
6	1934	8.2	4.94	7.80	63	9.0	18.7
	1935	13.8	6.63	6.22	63	7.2	14.2
	1938	9.3	3.96	6.55	65	13.9	32.3
	1939	20.8	7.35	7.89	62	5.1	12.6
	1940	15.0	5.68	4.28	63	7.9	10.4
	1941	7.9	3.62	7.93	66	11.6	15.8
	1942	25.4	9.45	7.41	61	6.9	8.95
	1943	12.9	2.38	10.12	63	8.9	9.70
7	1935	10.6	4.43	5.87	62	6.5	12.5
	1938	11.7	4.73	8.48	64	11.2	21.9
	1939	20.7	6.46	8.82	60	5.1	8.7
	1940	21.8	5.32	5.36	61	6.4	7.55
	1942	25.4	6.43	7.82	60	5.9	9.75
	1943	8.6	3.20	8.16	61	12.6	19.45
8	1934	16.7	6.16	8.24	60	5.1	1.8
	1939	29.4	7.43	10.05	60	3.4	5.8
	1942	25.9	8.97	7.95	60	5.9	2.2
	1943	20.5	3.75	7.63	62	8.4	3.30
9	1934	18.1	6.42	8.51	59	6.8	2.4
	1935	14.3	4.41	7.13	62	5.3	6.75
	1939	21.5	5.32	9.07	59	5.6	7.7
	1942	26.2	7.68	7.82	60	5.1	5.95
	1943	17.9	4.84	10.66	59	9.3	6.85

* No estimate is available.

The data of Table 1 leave much to be desired as to accuracy. Meteorological data taken at four to six points in each crop district can give at best a rough approximation of the conditions existing over the area as a whole, especially as regards rainfall. Hopkins (7) states that a given number of meteorological stations would provide a much less complete specification of precipitation than of air temperature within the area under consideration. Similarly root-rot data taken from 10 to 20 fields in a crop district represent a minimum sampling. Yield statistics likewise are subject to error. Inaccuracies of this type cannot be eliminated, but may be substantially reduced by the collection of additional data.

COMMON ROOT-ROT RATINGS AS ESTIMATES OF LOSSES IN YIELD

The ratings of Table 1 are in effect estimates of reduction in yields of wheat caused by common root rot. They are based on incidence and severity of the disease as found in annual surveys, and the effects of the various severity classes of lesions upon yields of grain. The disease ratings or estimates of loss range from 3.4 per cent in crop district 8 in 1939 to 17.8 per cent in crop district 3 in 1936. The simple average of the estimated losses for the 64 crop district-years of Table 1 is 8.8 per cent of the crop realized. Machacek (9) has estimated losses from common root rot in Manitoba to be 12.1 per cent of the crop over the three year period 1939 to 1941. His estimates were derived in essentially the same way as were the disease ratings of this paper.

Estimates of loss in yield of wheat caused by common root rot made in this way are open to criticism on a number of scores, some of which are indicated in the introduction to this paper. Perhaps the most serious one is that the diseased plants usually grow in intimate association with the relatively healthy plants used as a basis for comparison of yields. The healthy plants may be larger and yield more grain than the diseased ones, because they benefit by reduced competition from neighbouring diseased plants. If so, then any estimate of losses based on such a differential in yield would be inflated. This consideration may be partially or more than offset by an undetermined degree of lesioning present in the extensive root system or other portions of the plants used as the basis of comparison.

In an attempt to assess the effect of root rot on yields of grain, and at the same time avoid as many of these criticisms as possible, the methods of partial correlation and partial regression of yield on common root rot were employed. For these purposes, the common root-rot ratings of Table 1, instead of being considered estimates of loss, were taken as indices or relative measures of disease present in the crops. By the method of partial regression, taking into account variables such as precipitation, the relation between the root-rot ratings and yield could then be established.

THE RELATIONSHIP OF PRECIPITATION TO WHEAT YIELDS

There can be no doubt of the importance of rainfall to wheat production in Saskatchewan. It is a fact of common experience year after year that the yields of wheat are low when rainfall is low and they increase with increased rainfall. Hopkins (6) has made a statistical study of the effects

of rainfall on yields at successive periods and demonstrated that the distribution as well as amount of rainfall in the growing period has an important bearing on wheat yields.

In any study of the relation of water to yield, it is preferable to use data relating to the actual amount of water in the soil which is available or may become available to the plant during the growing season. Thus moisture conserved in the soil for many months in advance of the planting season undoubtedly influences yields, as well as moisture added to the soil by seasonal rainfall. However, data on the amount of water conserved in the soil are not available on a sufficient scale for the purposes of the present study, and rainfall data are all that can be obtained. Many factors influence the proportion of the rainfall that is conserved in the soil for plant use, such as condition, texture and composition of the soil, amount and distribution of rainfall, run-off, percolation, evaporation, and weed growth.

The correlations between rainfall and yield were investigated. For this purpose, the monthly precipitation data obtained from several stations within each crop district in Saskatchewan and reported (10, 11) in inches were used. Since some rainfall from the summer and fall preceding the crop year is conserved in summerfallow and stubble land, the rainfall data from July 1 of one year to July 31 of the succeeding year were considered. Rainfall between the ripening of the previous crop and the commencement of summerfallow operations probably is conserved to only a minor degree due to losses by weed growth, evaporation, etc. The data were grouped into four periods which may be designated as follows: fall rainfall (July, August, September, and October); winter precipitation (November, December, January, February, and March); spring rainfall (April and May); and summer rainfall (June and July). The distinction between April-May and June-July rainfall in the crop year was made since common root rot as it occurs in Saskatchewan develops very largely in the June-July period. Table 2 shows the simple correlations between the rainfall of the above four periods, singly and in combination, and wheat yields by crop districts over a period of 10 years.

TABLE 2.—SIMPLE CORRELATIONS BETWEEN RAINFALL AND WHEAT YIELDS

Periods	Coefficient of correlation
Fall	0.353
Winter	0.214
Spring	0.391
Summer	0.717
Fall and spring	0.466
Fall, spring and summer	0.781
Fall, winter, spring and summer	0.717

All the correlations in Table 2 are significant to the 1 per cent point except that between winter precipitation and yield. Since snowfall is believed to influence crop yields usually to only a slight extent, due to exceptional losses by run-off and evaporation, it will not be considered further in the present study. Barnes and Hopkins (1) found at Swift Current that all winter precipitation was lost in three years out of six. In three years when moisture was conserved, the amounts were respectively

0.66, 0.78, and 1.41 inches out of 5.7, 4.5, and 5.5 inches of precipitation by well-prepared summerfallowed land. Fall and spring rainfall influences yield significantly, the correlation coefficients 0.353 and 0.391 exceeding the 1 per cent level of significance. As there seems to be no good reason for separating the two periods of rainfall in a study of common root rot, the correlation coefficient of yield and the combined fall and spring rainfall, 0.466, will be used in the succeeding study and will be designated preseasonal rainfall. Similarly June and July, or summer, rainfall with a correlation coefficient of 0.717 will be used as distinct from preseasonal rainfall, since June-July rainfall has a definite relationship with common root rot (Table 3).

PRECIPITATION AND TEMPERATURE IN RELATION TO COMMON ROOT-ROT INCIDENCE

In Table 3 the simple correlation between common root rot and June-July rainfall is shown to be -0.632 and that between common root rot and air temperatures to be 0.550. It appears from this that increases in common root rot are associated with decreased amounts of summer rainfall and increased air temperatures. There is also an obvious relation between air temperatures and rainfall, the simple correlation in the present study being -0.622.

Considering first the relation of June-July rainfall to common root rot, the simple correlation -0.632 is reduced to -0.443 when air temperature is held constant. This is still a highly significant partial correlation, with 56 degrees of freedom. Apparently, considerable precipitation in June and July tends to suppress the development of common root-rot lesions on the underground parts of culms of wheat in Saskatchewan. On the other hand, light rainfall during these months, or possibly a considerable period of drought, appears to favour the involvement of these tissues by the common root-rot organisms.

The partial correlation between air temperatures in June and July and common root rot holding rainfall constant is 0.258, a substantial reduction from the simple correlation of 0.550. Since the value 0.258 with 56 degrees of freedom barely reaches the five per cent level of significance, more extensive data would be required to indicate a definite relationship between air temperature and common root-rot development. Probably a more precise measurement of the relation of temperature to common root-rot development would be obtained if sufficient data on soil temperature at seed level were available. It seems probable that when air temperatures are within the range 59° to 68° F. common root rot tends to increase with higher temperatures.

THE RELATIONSHIP OF COMMON ROOT ROT TO WHEAT YIELD AS INFLUENCED BY OTHER VARIABLES

Simple correlation coefficients between yield as the dependent variable and several independent variables, pre-seasonal rainfall, June-July rainfall, temperature, common root rot, and insect damage, are given in Table 3. In order to determine the effects on yield of any one of the independent factors, partial correlations were calculated from the simple correlations by the tabular method of solving normal equations, as outlined by Goulden (5). The partial correlations are shown in the latter half of Table 3.

TABLE 3.—SIMPLE AND PARTIAL CORRELATION COEFFICIENTS FROM DATA GIVEN IN TABLE 1

——	Yield	Insect damage	Common root rot	Air temperature	June-July rainfall
		Simple correlation coefficients			
Pre-seasonal rainfall	0.466*	−0.261	−0.183	−0.265	0.123
June-July rainfall	0.717*	−0.394*	−0.632*	−0.622*	
Air temperature	−0.705*	0.624*	0.550*		
Common root rot	−0.683*	0.422*			
Insect damage	−0.599*				
		Partial correlation coefficients			
Pre-seasonal rainfall	0.503*	0.044	0.125	−0.005	−0.296
June-July rainfall	0.469*	0.166	−0.207	−0.242	
Air temperature	−0.215	0.380*	0.061		
Common root rot	−0.349*	0.009			
Insect damage	−0.283				

Note.—Correlation coefficients marked with an asterisk exceed the one per cent level of significance.

The highly significant simple correlations between the independent variates and yield are reduced considerably when the partial correlations are taken, with the exception of pre-seasonal rainfall. The other variables, June-July rainfall, air temperature, common root rot, and insect damage are not significantly related to pre-seasonal rainfall. They, do, however, interact among themselves as shown by the simple correlations. Thus air temperature is negatively correlated with June-July rainfall and appears also to influence the incidence of common root rot and insect injury. Similarly June-July rainfall is negatively associated with common root rot and insect injury. Also, insect injury and common root-rot incidence appear to be positively correlated. Thus there are apparent interaction effects between the independent variables which when eliminated by the method of partial correlation reduce the apparent effects of certain variables on yield. For example, the simple negative correlation coefficient of −0.683 between common root rot and yield is reduced to −0.349 by the method of partial correlation. Both common root rot and yield, however, are subject to influence by June-July rainfall and air temperature. The relation between air temperature and common root rot and yield is further complicated by the influence of June-July rainfall on air temperatures. Blair (2), working with May-June precipitation, and June air temperature records and wheat yield data for North Dakota and South Dakota found relationships similar to those given above. His partial correlations between yield and May-June rainfall were positive. On the other hand, increases in air temperature in June apparently depressed yields of wheat, the partial correlations being −0.30 for North Dakota and −0.48 for South Dakota.

After eliminating the interaction effects of the several independent variables, it is apparent that there remain significant partial correlations between yield and the two sets of rainfall data and also the incidence of

common root rot. Thus it appears that variations in the infection of wheat by common root rot are related to yields of grain and may be instrumental in reducing yields to an appreciable degree.

In considering the regression of yield on common root rot, it would appear from these correlation studies that the independent variates, June-July rainfall, pre-seasonal rainfall, air temperature and insect injury are worthy of consideration.

THE PARTIAL REGRESSION OF WHEAT YIELD ON COMMON ROOT ROT

By making use of the statistical technique set forth by Fisher (4) for the study of regression with several independent variates, it is possible to derive the partial regressions of yield (Y) on June-July rainfall (x_1), pre-seasonal rainfall (x_2), air temperature (x_3), insect injury (x_4), and common root rot (x_5).

Beginning with June-July rainfall in Formula 1 below and including an additional independent variate successively, the following partial regression formulae may be set up:

$$Y = b_1x_1 \qquad 1$$
$$Y = b_1x_1 + b_2x_2 \qquad 2$$
$$Y = b_1x_1 + b_2x_2 + b_3x_3 \qquad 3$$
$$Y = b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 \qquad 4$$
$$Y = b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_5 \qquad 5$$

from which wheat yields (Y) may be calculated. The inclusion of any independent factor (x) with its corresponding partial regression value (b) in the equation may be justified if it results in a significantly improved fit of the calculated yield with the official estimate of yield.

Taking as working units 0.71 inches of June-July rainfall, 0.81 inches of pre-seasonal rainfall, 1° F. of air temperature, 5.5% estimated loss due to insect injury, 1.5 on the common root-rot disease rating scale, and 2.9 bushels per acre of yield, the following sums of squares and sums of products of deviations from the means were obtained:

	$S(y^2)$	354.17	$S(x_3^2)$	304.54	
	$S(x_1^2)$	306.03	$S(x_4^2)$	251.63	
	$S(x_2^2)$	262.31	$S(x_5^2)$	240.24	
$S(x_1y)$	+227.27	$S(x_1x_2)$	− 9.90	$S(x_2x_4)$	− 67.53
$S(x_2y)$	+106.81	$S(x_1x_3)$	−167.86	$S(x_2x_5)$	− 22.93
$S(x_3y)$	−206.92	$S(x_1x_4)$	− 97.51	$S(x_3x_4)$	+127.96
$S(x_4y)$	−184.07	$S(x_1x_5)$	−166.64	$S(x_3x_5)$	+139.24
$S(x_5y)$	−199.15	$S(x_2x_3)$	− 14.59	$S(x_4x_5)$	+ 97.66

Using equation 5, the partial regressions of yield on June-July rainfall (b_1), on pre-seasonal rainfall (b_2), on air temperature (b_3), on insect injury (b_4), and on common root rot (b_5) are obtained by the solution of the five simultaneous equations:

$$306.03b_1 - 9.90b_2 - 167.86b_3 - 97.51b_4 - 166.64b_5 = 227.27$$
$$-9.90b_1 + 262.31b_2 - 14.59b_3 - 67.53b_4 - 22.93b_5 = 106.81$$
$$-167.86b_1 - 14.59b_2 + 304.54b_3 + 127.96b_4 + 139.24b_5 = -206.92$$
$$-97.51b_1 - 67.53b_2 + 127.96b_3 + 251.63b_4 + 97.66b_5 = -184.07$$
$$-166.64b_1 - 22.93b_2 + 139.24b_3 + 97.66b_4 + 240.24b_5 = -199.15$$

The partial regression values with their standard deviations after converting from working units are $b_1 = 1.607 \pm 0.388$ bu. per acre for each inch of June-July rainfall, $b_2 = 1.120 \pm 0.283$ bu. per acre for each inch of pre-seasonal rainfall, $b_3 = -0.557 \pm 0.263$ bu. per acre for each increase of 1° F. in air temperature, $b_4 = -0.148 \pm 0.048$ bu. per acre for each 1 per cent of estimated insect injury, and $b_5 = -0.583 \pm 0.203$ bu. per acre for each increase of 1 unit in common root-rot disease rating. Applying the *t* test, the regression of yield on air temperature exceeds the 5 per cent level of significance, while all other regressions exceed the 1 per cent level.

Substituting the above partial regression values in Formula 5, the regression equation is

$$Y = 39.905 + 1.607x_1 + 1.120x_2 - 0.557x_3 - 0.148x_4 - 0.583x_5 .$$

Figure 1 illustrates the general agreement between yields calculated on the basis of this equation and the yields reported by the Secretary of Statistics,

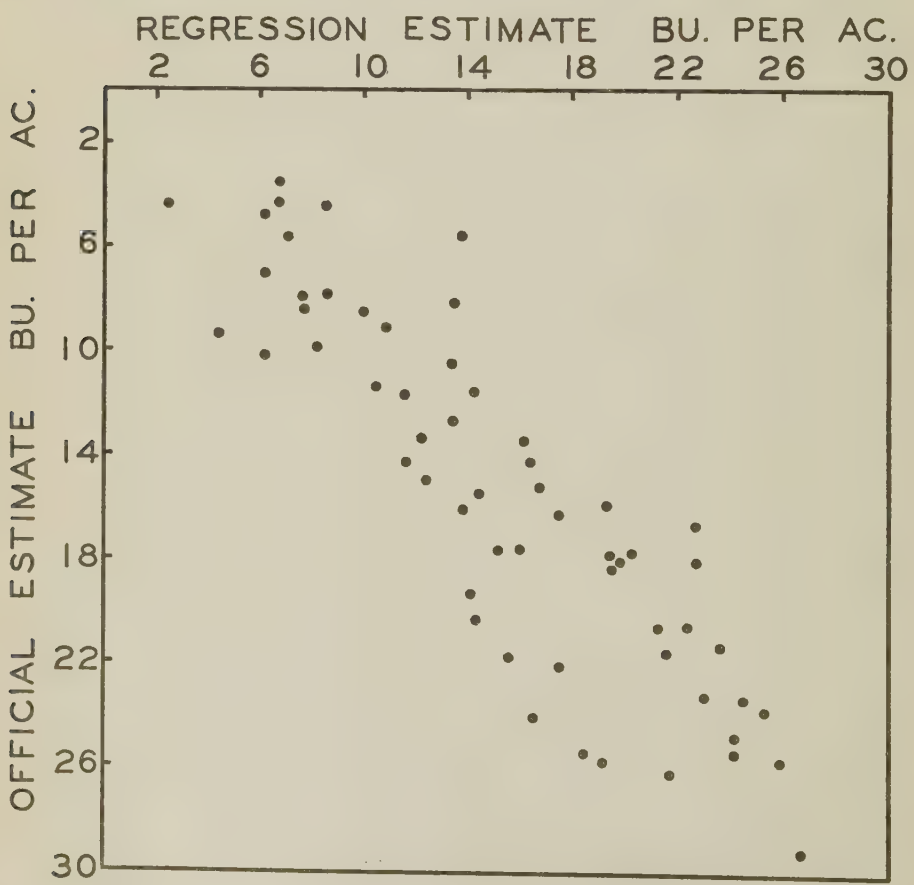

FIGURE 1. Dot diagram showing the degree of agreement between yields of wheat in bushels per acre by crop districts as reported officially and as calculated from partial regressions of yield on the independent variates, June-July rainfall, pre-seasonal precipitation, air temperatures for June and July, insect damage, and common root rot.

Saskatchewan Dept. of Agriculture (10, 11). Some factors influencing the yields which were not taken into account in the regression formula are discussed later.

The total sum of squares for yield, $S(y^2) = 354.17$, may be partitioned into portions related to the independent variables and a residual, $S(y-Y)$,[2] from equations of the form of

$$S(y-Y)^2 = S(y^2) - b_1S(x_1y) - b_2S(x_2y) - b_3S(x_3y) - b_4S(x_4y) - b_5S(x_5y)$$

and these may be set up in analyses of variance as in Table 4, using working units. The partition of the yield sum of squares is shown for each of equations 1 to 5.

TABLE 4.—ANALYSES OF VARIANCE IN YIELD ACCOUNTED FOR IN EQUATIONS 1 TO 5, MULTIPLE CORRELATIONS, AND PERCENTAGE OF YIELD VARIANCE ACCOUNTED FOR BY THE INDEPENDENT VARIATES

Source of variance	Equation number				
	1	2	3	4	5
		sums of squares			
June-July rainfall (x_1)	168.77*	172.02	128.36	117.73	89.43
Pre-season rainfall (x_2)		46.54	43.66	35.28	33.40
Air temperature (x_3)			72.12	50.61	39.75
Insect damage (x_4)				58.46	52.79
Common root rot (x_5)					60.12
Residuals (deviations from regressions)	185.40	135.61	110.03	92.09	78.68
Residual degrees of freedom	57	56	55	54	53
Residual mean squares	3.25	2.42	2.00	1.71	1.48
Multiple correlations	.690	.785	.830	.860	.882
Percentage of variance in yield accounted for	47.6	61.7	68.9	74.0	77.8

* All sums of squares for independent variates, with one degree of freedom each, exceed the 1 per cent level of significance.

The mean variance for residuals in equation 5 is 1.48 which, with 53 degrees of freedom, indicates that each of the five independent variates is related to a highly significant degree to the variance in yield. It is interesting that the variance in yield associated with common root rot is second only to that related to June-July rainfall.

The multiple correlation, R, a measure of the dependence of yield on the combined variation of the five independent variates, is clearly significant. The actual value of the multiple correlation in equation 5 is 0.8823. The proportion of the total variance in wheat yield accounted for in equation 5 is 77.8 per cent, leaving 22.2 per cent of the variance in yield ascribable to error and to factors not entering into the regression formula.

With the object of testing whether the use of common root-rot data in the partial regression formula has significantly increased the precision with which yields of wheat were calculated, Formulae 1, 2, 3, and 4, given above were also solved. Table 4 gives analyses of the variance of yield on the basis of partial regressions so obtained. Table 4 shows multiple correlations and the percentage of the variance in yield accounted for by the auccessive addition of factors in the partial regression formulae.

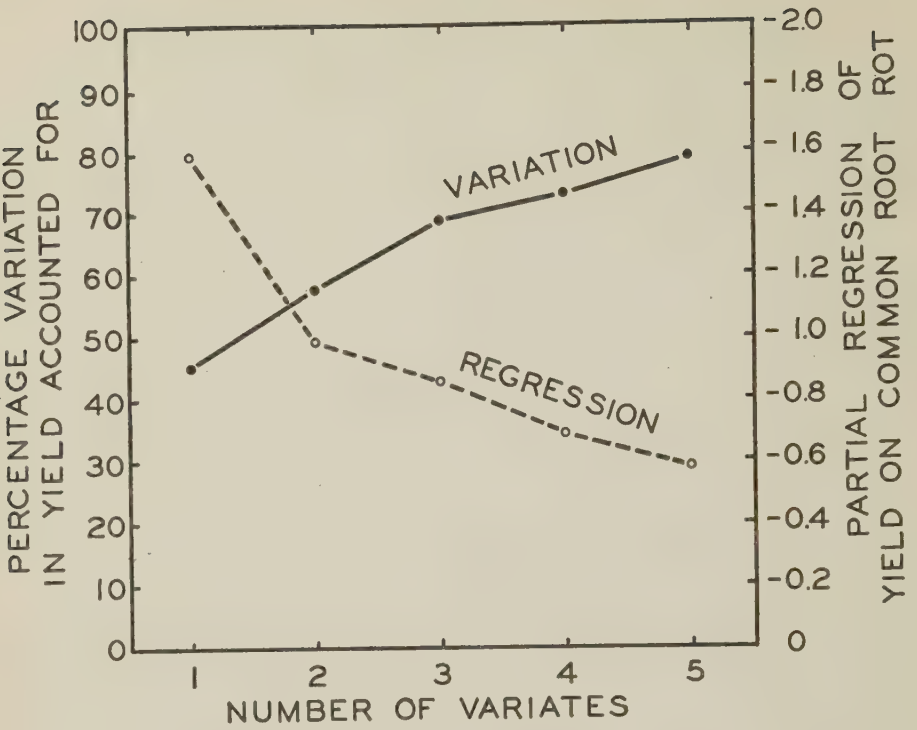

FIGURE 2. The influence of introducing additional variates into the regression equation upon the percentage variation in yield accounted for and the partial regression of wheat yields on common root rot. The independent variates were introduced in the following order: common root rot, June-July rainfall, pre-seasonal precipitation, air temperatures, and insect injury.

The successive inclusion of additional independent variates in the partial regression formulae has reduced the residual mean square from 3.25 to 1.48; increased the multiple correlations from 0.690 to 0.882; and has increased the percentage of the variance in yield accounted for from 47.6 per cent to 77.8 per cent. The partial regression of common root rot on yield has contributed to the increased adequacy of the regression formula to account for variance in yield as has each of the other factors considered. It appears therefore that common root rot bears an important relationship to yields of wheat.

The influence on the regression of yield on common root-rot ratings of taking into account successively the variates June-July rainfall, pre-seasonal rainfall, air temperatures and insect injury is indicated in Figure 2. The inclusion of other important factors concerned in yields of wheat into the regression formula might be expected to further reduce the partial regression of yield on common root rot. Consequently, estimates of losses in yield coincident with common root-rot infections would be somewhat reduced. At the same time the percentage variation in yield accounted for in the regression equation would be increased (Figure 2), and the residual variance consequently would be reduced. Thus, there should be an improved precision in the estimate of losses in yield associated with common root-rot incidence.

It may be of value to consider whether the increase from one regression equation to the next in the proportion of yield variance accounted for is significant as judged by the residual mean squares. Table 5 shows the increases in the sums of squares indicating the improvements in the formulae by successive addition of independent variates. Thus, the increase in the sums of squares from 262.08 to 275.49 = 13.41, resulting from the inclusion of common root rot, is highly significant compared to the residual mean square of 1.48 with 53 degrees of freedom.

TABLE 5.—ANALYSES OF VARIANCE APPLIED TO TEST SUCCESSIVE IMPROVEMENTS IN ESTIMATE OF YIELD GIVEN BY FORMULAE 1 TO 5

Equation	Added variable	Regression sums of squares			Residual	
		By formula	Increase	DF	Mean square	DF
1	June-July rainfall	168.77	—	—	3.25	57
2	Pre-season rainfall	218.56	49.79*	1	2.42	56
3	Air temperature	244.14	25.58*	1	2.00	55
4	Insect injury	262.08	17.94*	1	1.71	54
5	Common root rot	275.49	13.41*	1	1.48	53

* Significance exceeds the 1 per cent level.

From this study it appears that common root rot has an important relationship to wheat yields in Saskatchewan. The partial regression of yield on common root rot, -0.583 with a standard error of ± 0.203 bushels per acre for each increase of one unit in common root-rot disease rating, indicates a substantial reduction in yield of wheat associated with root rot. From the average disease rating in this study of 8.81, an average loss of 5.14 ± 1.79 bushels per acre may be calculated. The 95 per cent fiducial limits within which the actual average loss may be expected to occur are 1.55 to 8.73 bushels per acre.

DISCUSSION

The data given in this paper support the opinion held by plant pathologists that common root rot depresses yields of wheat. The statistical methods of partial correlation and partial regression used in the reduction of these data do not constitute proof of causal relationships between the disease and loss in yield. However, the harmful effects of the common root-rot organisms on wheat plants have been demonstrated over and over, not only in pure culture in the laboratory, but also in the greenhouse and the field. The statistical procedures do, nevertheless, demonstrate that a certain relationship between the occurrence of the disease and crop yield exists and give a measure of this relation. It appears justifiable, therefore, to use these procedures in determining the effect of common root rot on wheat yields.

The simple correlation, -0.683, found between common root rot and yield, indicates that decreases in yield and increases in common root rot are associated and that when factors such as rainfall, temperature and insect injury, which influence yields independently and some of which are related

to the incidence of common root rot, were held constant by the method of partial correlation there remained a significant association between the disease and yields of -0.349. While this value does not indicate a very close dependence of yield on common root-rot incidence, it is probably as high a value as could be expected where wheat yields are subject to many variations as in Saskatchewan.

The loss from common root rot in Saskatchewan over the past ten years of 5.14 bushels per acre, as determined in this study, is very large in the aggregate and amounts to about one-third of the yields actually harvested. This estimate of loss, however, is subject to a large standard error since some 22 per cent of the variance in yield is unaccounted for. A close examination of Table 4 indicates that in the case of June-July rainfall the successive addition of other variates into the equation has resulted in substantial reductions of the proportion of the variance in yield ascribable to June-July rainfall. It seems probable that if all relevant factors could be used in the equation for predicting yields, some at least would be so related to common root rot as to reduce the above estimate of loss. A factor which may be so related to both yield and common root rot is the general level of soil fertility and its availability to the crop. Barnes and Hopkins (1) have shown that substantial increases in yields of wheat may be obtained on plots treated with phosphatic fertilizers without depleting the reserve of soil moisture remaining in the soil as compared with the reserve in untreated control plots. Apparently the plants on phosphate-treated plots were more efficient in the use of water than were plants on the untreated plots. Similarly Kiesselbach (8) has shown that the water requirements of corn are substantially lower in fertile than in an infertile soil. In the case of wheat, this interaction of fertility with moisture, besides increasing yields directly, may also indirectly increase them by reducing the injury caused by the root-rotting fungi.

Certain factors, not taken into consideration in this work, have undoubtedly had large influence on yields of wheat. While certain data were excluded due to exceptionally large losses in some years in eastern parts of the province from stem rust, certain other data were included where rust losses ranged up to probably 25 per cent. Some of these can be picked out in Figure 1 as having reduced actual yields as compared with calculated yields. Similarly, while in general there did not appear to be a statistically significant relation between winter precipitation and yields, winter snows may sometimes be important. It has been found at Swift Current, by Barnes and Hopkins (1), that snow adds little if any moisture to the soil. However, the same finding may not hold for certain other sections of the province, such as crop districts 5, 8, and 9. Some of the wider variations in Figure 1 between actual yields and yields calculated from the regression equations appear to be explainable by unusually heavy snowfall in the preceding winter months.

A factor of some considerable importance, as affecting yields, is the proportion of wheat on summerfallowed land, as opposed to spring or fall worked stubble land. Such statistics were not available to the author. However, on the assumption that wheat is sown on summerfallowed land to the fullest extent possible, it may be deduced from data given by the

Provincial Secretary of Statistics (10, 11) that 55 to 70 per cent of wheat was on fallowed land in the years 1934 to 1940. In 1941, the proportion rose to 83 per cent while in 1942 and 1943 the acreages of prepared summerfallow exceed wheat acreages by 12 per cent and 14 per cent, respectively. It may reasonably be assumed that nearly 100 per cent of all wheat in these two seasons was seeded on land summerfallowed the previous year. Thus actual yields on a crop district basis in these two years are not strictly comparable with yields from earlier years, and the regression of yield on the various independent factors as determined in this paper is no doubt influenced.

When all the important variables correlated with both yield of wheat and common root rot are introduced into the regression equation it is not impossible that the effect of the disease upon yield may prove to be nil. A final conclusion as to the effect of common root rot on wheat yields would therefore depend upon introducing into the regression equation all of the important factors with which both yield and common root rot are correlated.

These considerations notwithstanding it appears probable that yields of wheat are reduced appreciably by common root rot. The 95 per cent fiducial limits of the estimate of 5.14 bushels per acre loss are 1.55 and 8.73 bushels per acre. The lower limit would in itself constitute a large aggregate loss, being about 10 per cent of the harvested yields of wheat. This figure is not far from estimates (9) of annual losses of 9 per cent in Saskatchewan and 5 per cent in Manitoba, or from the estimates made by Machacek in Manitoba to which reference is made above.

SUMMARY

An attempt was made to measure the relation of common root rot to wheat yields in Saskatchewan using the methods of correlation and partial regression. Since a number of factors interact with common root rot in influencing yields, data were collected for rainfall of the growing season (June and July), for air temperatures of the same period, for pre-seasonal rainfall excluding winter precipitation, and for insect damage. The statistics were assembled on the basis of the nine crop districts of the province as units, over a period of ten crop seasons.

The simple average common root-rot rating over the periods and areas covered by the data is 8.8. This may be taken as an estimate of the percentage loss. Some of the weaknesses of such an estimate are discussed.

The coefficient of correlation between rainfall in the June-July period and wheat yield was 0.717, while that between pre-seasonal rainfall and wheat yield was 0.466.

Considerable precipitation during June and July tends to suppress common root-rot lesions, and light rainfall appears to favour increases in incidence and degree of severity of lesions.

Pre-seasonal rainfall and June-July rainfall favoured wheat yields as shown by the partial correlations 0.503 and 0.469, respectively. Depressing effects on yields were indicated by the partial correlations of -0.215, -0.349, and -0.283 between yields and air temperature, common root rot and insect damage, respectively.

Partial regressions of wheat yields on five independent variates accounted for 77.8 per cent of the variance in wheat yields. Each of the five independent variates were related to wheat yields in a highly significant degree. The variance in yield associated with common root rot was second only to that portion related to June-July rainfall.

On the basis of the partial regression of wheat yields on common root-rot ratings, it is estimated that the average loss associated with this factor is 5.14 bushels per acre, with 95 per cent fiducial limits of 1.55 and 8.73 bushels per acre. Some factors which may reduce this estimate a discussed.

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THE OCCURRENCE OF BACTERIA IN NORMAL POTATO PLANTS AND LEGUMES[1]

G. B. SANFORD[2]

Dominion Laboratory of Plant Pathology, Edmonton, Alberta

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During 1939, in preliminary studies of the possible causes of an apparently new potato disease designated as purple dwarf (3), bacteria were found to inhabit not only the stems, stolons, and roots of the affected plants, but those of healthy plants as well. In contrast, fungi were isolated only sporadically from the same areas, and in practically all cases their presence was associated with mechanical injuries or lesions apparently caused by *Rhizoctonia Solani*. The occurrence of bacteria in normal plants was unexpected, and, therefore, suggested further study. During each season since 1939, a large number of isolations have been made from the general vascular region of the stems of healthy potato plants of various ages, as well as from the stems of *Phaseolus vulgaris* and the tap roots of *Medicago* spp. and *Melilotus* spp.

MATERIALS AND METHODS

In view of the obvious hazards in attempting to demonstrate the presence of living bacteria in healthy plant tissue, it seemed that the most reliable method would be to cut bits of tissue from the aseptically bared stele and to incubate them on nutrient agar in Petri plates. With this technique it would not be necessary to attempt to sterilize completely the epidermal layer, since this would be stripped off. Moreover, if the intact specimen is carefully washed and surface disinfected, its epidermal layer will remain turgid, in which condition it can be quickly removed in wide strips, with practically no danger of contaminating the stele.

Every possible precaution was taken to prevent contamination during the preparation of the plant specimens selected and in plating from them. Within an hour or so after the plants were removed from the soil, platings were made from the lower four inches of the stems of potato and bean, and from the upper four inches of the tap root of alfalfa and sweet clover. Each stem or root selected for study was dipped for about one-half minute in methyl alcohol and the excess alcohol on the specimen burned off. One set of scalpels and forceps was reserved for cutting and pulling off the epidermis and another scalpel for cutting the sections from the bared stele. They were flamed frequently and thoroughly when in use and kept in alcohol when not employed.

Choosing a space free from roots, and one-half to two inches above the base of the stem, or below the crown of the tap roots, as the case may be, the epidermal layer was quickly peeled off under aseptic conditions, and thin cross-sections were cut from the general vascular region of the stele at points which had not been touched previously by scalpel or forceps. These were immediately picked off the scalpel with sterile forceps by an assistant and plated. Five of these bits of tissue, each from a different location,

[1] Contribution No. 922, Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Pathologist-in-Charge.

were plated from each of ten or more different stems or roots in all tests made. It required from 15-20 seconds to cut and plate the sections from each specimen.

The plates were placed on a bench at room temperature to incubate during a period of about six days, which was usually adequate.

As a partial check on the technique used, and an indication of the degree of air-borne contamination, bits of tissue from the central area of a young potato tuber were plated, using the same general procedure as employed with the test material. For each test there were ten of these control plates, each containing five bits of tissue.

RESULTS

The comparative data presented in Table 1 are from six separate tests made at different dates, and are reasonably typical of the results of a large number of other tests made during this study.

The percentage of potato stems which were negative for bacteria after five or six days of incubation in Tests A, B, C, D, E, and F, respectively, were 16.6, 15, 0, 8.3, 0, and 53.5; the respective ages of the stems being 60, 90, 90, 61, 42, and 22 days. There was no obvious explanation for the relatively higher percentage of stems that were negative in Test F, which stems were from uncut tubers grown in sand and only 22 days old.

Although these data were based on the incubation of bits of tissue cut from the stele of potato stems about two inches above the base, bacteria were also obtained at higher levels.

Bacterial colonies usually developed from most of the plated stem or root sections by the second or third day. Other sections remained apparently negative until later and occasionally a few of them were still negative at the end of 15 days. In contrast, no bacterial colonies developed from the bits of tissue plated from the inside of young potato tubers used for controls. This fact and the comparatively rare occurrence of either bacterial or fungal contaminations from obviously air-borne sources in the plates, when taken together, are considered satisfactory evidence that the bacterial growth obtained from the test tissue indicated was not a result of contamination from air-borne or other sources, but of living bacteria from within the stele.

A high percentage of the sections of tissue cut from the stele of tap roots of alfalfa and sweet clover, and from the stems of beans, also yielded several types of bacteria in each of the three tests made.

Avenue of Entrance

It would appear from the results in Table 2 that sterilizing the soil with steam, disinfecting the sets, or planting them whole or cut, made no difference in the incidence of bacteria in the potato stems. Also, it is obvious that these results do not offer a clue as to the location of the bacteria in the stele or indicate whether the bacteria entered the stem by way of the root system or the set, or both. It will be recalled that, in general, the young, healthy tubers used for the controls in this study were apparently sterile. On the other hand, tissue from mature tubers was not

TABLE 1.—RESULTS FROM ASEPTICALLY CULTURING INSIDE WOODY TISSUE OF APPARENTLY HEALTHY POTATO STEMS

Test	Source	Sets	Age of stems days	Stems tested, No.	Stems No.	Sections* positive for bacteria, %	Incubation period days	Bacterial types
A	Pots	Cut	60	30			5	Mixed
					12	100		
					4	80		
					3	60		
					2	40		
					4	20		
					5	0		
(Control tuber)						0		
B	Field	Cut	90	20			5	Mixed, but large colonies of fast growing types predominated
					17	100		
					3	0		
(Control tuber)						0		Mixed
C	Field	Cut	90	20			6	
					18	100		
					1	80		
					1	20		
(Control tuber)						0		
D	Pots	Whole	61	24			6	Mixed
					16	100		
					1	80		
					1	60		
					4	40		
					2	0		
(Control tuber)						6		
E	Pots	Cut	42	23	23	100	6	Mixed
F†	Pots	Whole	22	15			6	Mixed
					1	100		
					2	80		
					2	60		
					1	40		
					1	20		
					8	0		
(Control tuber for E and F)						0		

* Based on 5 sections per stem.
† Grown in sand.

used for control material because it was found at the beginning of the study that bacteria were often present in the region of the vascular ring, especially in the stem end (4). Tyner (5) concluded that *Corynebacterium sepedonicum* (Spieck. and Kott.) Skaptason and Burkholder, may enter the potato stem by way of the root system.

Types of Bacteria

It was quite obvious from the general appearance of the bacterial colonies obtained from the stele of the stems of potato and bean, and from the stele of the tap roots of alfalfa and sweet clover, that often several kinds of bacteria were present (Tables 1 and 2). A majority of the sections

TABLE 2.—INCIDENCE OF BACTERIA IN POTATO STEMS 28 DAYS OLD, AS AFFECTED BY CUTTING OR TREATING THE SETS, AND BY STERILIZING THE SOIL WITH STEAM

Sets	Soil	Treatment of sets	Results* from 50 sections		Bacterial types
			Positive, number	Negative, number	
Cut	Sterilized	Nil	47	3	Mixed
Whole	Sterilized	Nil	38	12	Mixed
Cut	Sterilized	Yes†	44	6	Mixed
Whole	Natural	Nil	50	0	Mixed
Cut	Natural	Nil	43	7	Mixed
Cut	Natural	Yes†	41	9	Mixed
(Control tuber)			2	48	

* Based on plating 5 sections from each of 10 stems, incubated 5 days.
† Sets immersed 8 minutes in 1-1,000 mercuric chloride solution + 1% by volume of hydrochloric acid.

from most specimens, and especially those from the older field plants, yielded what superficially appeared to be one kind of bacteria, but dilution cultures made from these colonies often revealed the presence of two or more kinds, differing not only in size but in reaction to Gram's stain. One of these isolates, commonly present in the older potato stems, was identified as *Bacterium radiobacter* by Dr. A. G. Lochhead, Dominion Bacteriologist, Gttawa. The other isolates have not been identified yet.

DISCUSSION AND CONCLUSIONS

The results presented in this paper indicate that several kinds of bacteria may commonly inhabit the stele of apparently healthy stems of potato and garden beans, and the tap roots of alfalfa and sweet clover, as well as the interior of mature potato tubers. It is believed that the plate culture method used to demonstrate this phenomenon was not only satisfactory, but much more reliable than microscopic technique. Moreover, the fact that the tissue cultured was from the aseptically bared stele, and did not include the epidermis, eliminates natural concern as to whether the latter has been completely sterilized by a treatment.

That non-pathogenic bacteria or fungi may be present as symbionts in apparently healthy tissue of certain plants without producing obviously detrimental effects on the host is certainly not novel, in view of the many cases on record. On the other hand, the generally accepted idea has been that the inside tissue of most healthy plants is sterile. Possibly this is still true for many plants but not for others, which suggests the need for further careful study, with special reference to the identification of the symbionts, their location, relative abundance at different stages of maturity, and particularly their effect on the physiology of the host.

Since this report was written, two relevant papers (1, 2), each with a review of pertinent literature, were brought to my attention, and, therefore, a brief reference to their contents is added here. Hennig and Villforth (1)

studied 28 different healthy plants and found bacteria (mostly spore formers) in all parts (leaves, twigs, stems, and roots) of all plants at all stages. Their technique was to immerse the parts in a 1.5% bromine solution, rinse them in sterile water, aseptically cut out sections and incubate these in a nutrient solution. Marcus (2) cultured the inner sound tissue of seeds and fruits of a number of different plants and obtained fungi, yeasts, or bacteria from some kinds but found others sterile.

SUMMARY

In many separate tests, made during several successive years beginning 1939, thin sections, containing vascular elements, were aseptically cut from the stele of apparently healthy stems of *Solanum tuberosum* and cultured on potato dextrose agar. Evidence obtained from these tests indicates that the stele of stems of normal potato plants may harbour a mixed bacterial flora. Similar evidence was also obtained with respect to the tap roots of *Medicago* spp., *Melilotus* spp. and the stems of *Phaseolus vulgaris*.

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A NEW METHOD OF HYACINTH PROPAGATION INVOLVING ADDITIONAL WOUNDING, THE USE OF FUNGICIDAL DUSTS, AND A GROWTH PROMOTING SUBSTANCE[1]

By J. E. Bosher[2] and W. Newton[3]

Dominion Laboratory of Plant Pathology, Saanichton, B.C.

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In the propagation of hyacinth, the well-known method of "scooping" or "scoring" the bulbs to induce bulblet formation also creates favourable conditions for the entrance of fungi that promote bulb rots. In British Columbia, an unidentified species of *Penicillium* appears to be the chief cause of bulb decay in the propagation of hyacinth bulbs. The development of the fungus appears to be inhibited by the common practice of placing the mother bulbs in moist peat or peat and sand immediately after they are "scooped" or "scored"; nevertheless, ample evidence has been obtained that the control of bulb rot is the chief factor in the successful propagation of hyacinth bulbs.

Preliminary experiments indicated that the losses through the rotting of the bulbs can be reduced greatly by dusting the wounded surfaces with Arasan. These preliminary experiments suggested the development of a new method of propagation based upon the protection of the wounded bulb surfaces with a suitable fungicide, the wounding of the mother bulb tissue to a greater degree than by "scooping" or "scoring" to take advantage of the well-known relationship between the amount of wounding and bulblet formation, and the separation of the wounded scale tissue from the mother bulb to avoid the spread of rot throughout the mother bulb.

THE PROPAGATION METHOD

In the new method as developed, the mother bulbs are cut to a depth of three or four scales immediately above and around the base. The scales are then cut vertically into sections measuring approximately one inch wide at the base. A large bulb will produce up to forty such sections. The residual core, consisting of three or four scales surrounding the bud or central shoot with attached base, is not utilized in the production of bulblets. It is planted in the field, and, under favourable growing conditions, will produce a bulb of the original size in a single year. After the scales are dusted with a fungicidal dust they are maintained in moist peat or moist peat and clean sand for three months in a greenhouse or incubator at a temperature of 78-80° F. They are then transplanted into peat or peat and sand overlying a rich compost in a greenhouse or protected cold frame. Protection is essential, for many of the bulblets produce leaves, and if these are destroyed bulblet growth is materially reduced. The bulblets are harvested as soon as the foliage dies down. Great care in harvesting must be exercised if the loss of a significant fraction of the small bulblets is to be avoided. Screening the surface material through a quarter-inch mesh is recommended.

[1] Contribution No. 917 from the Division of Botany and Plant Pathology, Dominion Dept. of Agriculture, Ottawa, Canada.
[2] Technician, Grade 2, Dominion Laboratory of Plant Pathology, Saanichton, B.C.
[3] Officer-in-Charge, Dominion Laboratory of Plant Pathology, Saanichton, B.C.

THE INFLUENCE OF ARASAN AND NAPHTHALENEACETIC ACID ON BULBLET FORMATION

In the preliminary experiment the absence of visible growth of the *Penicillium* sp. indicated that Arasan was effective in preventing rot. A more extensive experiment was conducted to determine the influence of Arasan alone and in combination with naphthalenacetic acid, a growth-promoting substance, for there was the possibility that in spite of its effectiveness as a fungicide, Arasan might inhibit bulblet formation. There was also a probability that bulblet formation might be stimulated by naphthaleneacetic acid.

The dust containing the growth promoting substance was prepared by mixing with every 5 gm. of Arasan dust 1 ml. of a naphthaleneacetic acid solution containing 100 ppm. The Arasan and the Arasan-naphthaleneacetic acid dusts were applied by shaking the scale sections in a paper bag containing an excess of the dust.

To ascertain whether or not infection occurs shortly after the scales are cut, two lots of 22 scales were dusted immediately after they were cut, and two duplicate lots were dusted 24 hours later. Two lots of 22 scales were not treated, to serve as the controls. The benefit of Arasan, the greater benefit of the Arasan-naphthaleneacetic acid treatments, and the advantage of dusting the scales immediately after they are cut, are indicated by the data in Table 1. In the data the stage of bulblet development is reflected by the number of bulblets with leaves and the average leaf lengths. Bulblets that carry leaves are in an advanced stage of development.

THE INFLUENCE OF FUNGICIDAL DUSTS ON FUNGUS GROWTH AND BULBLET FORMATION

The influence of fungicidal dusts on fungus growth and bulblet formation was studied by applying each dust to a lot of twenty scales and then placing them on the surface of moist peat in flats covered with wax paper. The number in each lot was noted that bore evidence of the *Penicillium* sp. or other fungus growth after seven, nine, twelve, and fourteen days. At

TABLE 1.—THE INFLUENCE OF ARASAN AND OF ARASAN AND NAPHTHALENEACETIC ACID IN COMBINATION ON BULBLET FORMATION

Treatment	Time of application	Number of scales	Number of bulblets	Bulblets with leaves	
				Number	Av. leaf length
					inches
Arasan	After cutting	22	51	25	1.6
Arasan and naph. acid	After cutting	22	62	35	2.2
No treatment		22	34	24	1.6
Error 5%		—	6	7	0.4
Arasan	24 hrs. after cutting	22	44	14	1.1
Arasan and naph. acid	24 hrs. after cutting	22	58	35	2.0
No treatment		22	29	8	2.2
Error 5%		—	5	7	0.4

the end of the fourteen-day period the severity of the fungus infection on each scale was noted and was recorded for each lot as 0-10. After 80 days the number of bulblets on each lot of scales was recorded. The records are given in Table 2.

TABLE 2.—THE INFLUENCE OF FUNGICIDAL DUSTS ON FUNGUS GROWTH AND ON BULBLET FORMATION

Dust	Number of scales	Number of scales bearing fungus growth after days				Severity of infection	Number of bulblets
		7	9	12	14	0-10	
Spergon	20	0	0	0	0	0	34
Arasan	20	0	0	5	5	1	23
Nomersan	20	0	0	0	0	0	19
Fermate	20	0	19	20	20	9	16
Yellow cuprous oxide	20	0	0	2	2	1	2
Dithane	20	0	4	12	12	8*	0
None	20	20	20	20	20	10	3
Error 5%							8

* On the scales treated with Arasan, a *Rhizopus* sp. in addition to the *Pencillium* sp. was conspicuous.

Fungus growth was prevented from developing on the scales by dusting them with Spergon and Nomersan, and it was greatly inhibited by Arasan and by yellow cuprous oxide. On the other hand, fungus growth was abundant on the scales dusted with Fermate and Dithane. A *Penicillium* sp. was the only conspicuous fungus that developed on the scales, except on those dusted with Dithane, where, in addition, a *Rhizopus* sp. was conspicuous.

A greater number of bulblets developed on the scales dusted with Spergon than on those dusted with the other fungicides. However, large numbers of bulblets developed on the scales dusted with Arasan, Nomersan, and Fermate. The high yield of bulblets by scales dusted with Fermate was unexpected, for the fungicide failed to check the development of conspicuous fungus growth. Bulblet formation was small on the untreated scales and on those dusted with Dithane and yellow cuprous oxide. The small production of bulblets by the scales treated with yellow cuprous oxide is of special interest, for this fungicide appeared to check satisfactorily the *Penicillium* sp. from entering the tissue of the scales.

DISCUSSION

The new method of hyacinth propagation is particularly economical in the propagation of rare varieties and new varieties from seedlings largely because the risk is lowered of losing valuable variety lines through the replanting under field conditions of the residual bulb cores. These cores when planted under favourable field conditions seldom fail to regain their original size in a single season. Furthermore, the scales that are removed therefrom appear to produce a quantity of bulblets at least equal to the quantity produced by mother bulbs of corresponding size that had been

"scooped" or "scored" and otherwise handled in accord with standard propagation methods. The dusting of the scales and the residual bulb cores with Spergon or Arasan is an essential part of the new propagation method to offset the losses through rot. By following the new propagation method, more bulb tissue is cut than by following standard propagation practices with the consequent need of greater protection against the entrance through the wounds of rot-producing organisms. The inclusion of naphthaleneacetic acid in the fungicidal dust is also a part of the new method when the objective is to secure a maximum yield of bulblets.

SUMMARY

A new method of propagating hyacinth bulbs is described. The mother bulbs are cut around the base to a depth of three or four scales. To the depth of the cut the scales are removed and are cut longitudinally into sections of approximately one-inch in width. These are treated with a fungicidal dust and maintained in moist peat or peat and sand for three months at a temperature of 78-80° F. The residual cores are treated also with a fungicidal dust and are replanted under field conditions immediately after the scales are removed.

Scales dusted with Arasan produced a much greater number of bulblets than untreated. Production was increased further by including a trace of naphthaleneacetic acid with the Arasan dust. Scales dusted with Spergon produced a greater yield of bulblets than those dusted with other fungicides. Scales dusted with Arasan, Nomersan, and Fermate also were prolific, and with the exception of Fermate, these dusts, including Spergon, appeared to check satisfactorily the fungus growth on the scales. Dithane and yellow cuprous oxide failed to exert beneficial effects. Dithane allowed a *Rhizopus* sp. in addition to a *Penicillium* sp. to develop. Yellow cuprous oxide almost completely prevented bulblet formation, although this dust appeared to check the development of fungus growth on the scales.

The residual cores of the original mother bulbs when field planted usually produced in a single season new bulbs as large as the originals.

COMPARATIVE EFFECT OF KEEPING SOIL UNDER CONTINUOUS CULTIVATION AND KEEPING IT IN CONTINUOUS GRASS[1]

H. J. Atkinson[2] and L. E. Wright[3]

Division of Chemistry, Science Service, Ottawa

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The maintenance of a satisfactory level of soil fertility is one of the major problems in agriculture. It is a generally accepted fact that newly cultivated soils produce better crops than the same soils after many years of continuous cultivation. In other words, cultivation tends to reduce the level of fertility of most soils as measured by crop producing power and also by physical properties and chemical constitution, particularly the content of nitrogen and organic matter, and exchangeable bases. To counteract these trends, certain management practices are employed in an endeavour to maintain the productivity. Such practices include a suitable crop rotation, usually one containing a legume, addition of organic matter in the form of barnyard manure, green manures, etc., and the removal from cultivation for a period of years.

Many references can be found in the literature to support these statements. Russell (9) in his book "Soil Conditions and Plant Growth" points out that "directly ploughing and cultivation operations begin, great losses of nitrogen set in". On the other hand, he states that "experiment has shown that soil gains nitrogen and organic matter when it is allowed to remain undisturbed and covered with unharvested vegetation as in natural conditions". Shutt (10) in 1925 found that the grain-fallow rotation followed in Western Canada was "destructive of the soil's organic matter with a concomitant loss of nitrogen". He also observed that "the systematic and periodic putting of the land in sod will serve to maintain the soil's nitrogen and humus". Among more recent papers which compare the nitrogen and organic matter contents of soil samples from adjacent virgin and cultivated soil may be mentioned those by Dorman (2) of Michigan, Giddens and Garman (3) of Georgia and Newton, Wyatt and Brown (8) in Alberta. All show considerable losses in these constituents over a period of years. The effect of cropping and crop rotations has been discussed by Greaves and Hirst (4) of Utah, by Johnston *et al.* (6) of Iowa, and by Stauffer, Muckenhirn and Odell (11) with reference to the Morrow Plots at Urbana, Illinois where the same cropping system was followed from 1904 onwards.

The removal or retirement of soil from cultivation and keeping it in sod has also proved beneficial. Hall (5) in 1905 found that, in two fields of the Rothamsted Experimental Farm where the land was allowed to run wild and no crops were grown for over 20 years, the carbon and nitrogen content of the surface soil increased considerably. In Kentucky, Karraker (7) reported on the effect of keeping plots (*a*) bare by scraping, (*b*) in continuous bluegrass, and (*c*) in continuous bluegrass plus white clover. Over a period of about nine years, the bare plot lost 360 lb. N per acre, the

[1] Scientific contribution No. 141 from the Division of Chemistry, Science Service.
[2] Chemist, Division of Chemistry, Science Service, Central Experimental Farm, Ottawa.
[3] Chemist-in-charge, Soils and Fertilizer Unit, Division of Chemistry, Science Service, Central Experimental Farm, Ottawa.

TABLE 1.—CHEMICAL DETERMINATIONS ON SOIL SAMPLES DIVISION OF CHEMISTRY PLOTS

(Average of 24 samples)

Year of collection	pH	$\%H_2O$	% Loss on ignition	% N	% ex. CaO	% ex. MgO	% ex. K_2O	p.p.m. readily soluble P_2O_5
1931	6.5	0.78	2.99	0.12	0.143	0.011	0.010	161
1945 (old area)	5.8	0.61	2.43	0.09	0.088	0.004	0.010	144
1945 (new area)	6.1	1.13	4.77	0.18	0.186	0.012	0.010	217

bluegrass plot 113 lb. N per acre while the bluegrass-clover plot gained 172 lb. N per acre. At the end of the period, a crop of Sudan grass was grown on all plots and the smallest yield was obtained on the plot that was kept bare, with the largest yield on the bluegrass-clover plot.

In Pennsylvania, on the Jordan Soil Fertility Plots, six grass roadways between plots were in permanent grass from 1867 to 1944, and received no treatment. When samples were taken at the end of that period, it was found (12) "that in every one of twenty comparisons the grassland soils have maintained a nitrogen and organic matter level considerably above that of the plot soils".

The present fertilizer area of the Division of Chemistry, Science Service, at the Experimental Farm, Ottawa, is located on a soil type that closely resembles Uplands sand. It was taken over by the Division in 1931. The eastern part, which will be referred to as the "old area", has been kept in almost continuous cultivation since that time, certain ranges being occasionally sown to grain for one year, followed by hoed crops grown in experimental plots the next year. The western part was not cultivated for many years but the annual crop of grass containing some alfalfa was cut once a year and removed. In the fall of 1944, this area, which will be called the "new area", was ploughed and disked, and it was laid out in experimental plots in the spring of 1945.

In the early summer of 1945, soil samples were taken from both old and new areas in connection with experiments being conducted thereon. Since certain soil samples had been taken in 1931 which represented soil conditions at that time, it was felt that here was an opportunity of studying the effect of continuous cultivation and also of continuous grass on certain soil constituents. The determinations that were made on the soil samples collected in 1945 were pH, hygroscopic moisture, loss on ignition, nitrogen, exchangeable calcium, exchangeable magnesium, exchangeable potassium and readily soluble phosphorus. The same determinations were made on 24 samples from the 1931 collection selected so as to give a general picture of conditions at that time and, although they all came from the present "old area", there is every reason to believe that conditions in both areas, which are adjacent to each other and were previously cropped with the same rotation, were very similar at that time.

The average values of the aforementioned determinations for the 24 samples collected in 1931, for 24 samples from the old area and 24 samples from the new area collected in 1945, are presented in Table 1. It can be

TABLE 2.—CROP YIELDS FROM OLD AND NEW AREAS, DIVISION OF CHEMISTRY PLOTS, 1945

	Tomatoes (bu./acre)	Potatoes (bu./acre)
Old area—average of 24 plots	266	130
New area—average of 24 plots	581	271

readily seen that considerable changes in soil composition have taken place. Continuous cultivation has resulted in a pronounced decrease in loss on ignition and nitrogen, and in reaction (pH value), exchangeable calcium and exchangeable magnesium. There has been, however, no change in the exchangeable potassium content and no very significant change in the content of readily soluble phosphorus. This is not surprising since these constituents have been added in fertilizer applications to the old area in the intervening years. Nitrogen also has been added but in amounts so small that they could not be expected to affect the quantity of total nitrogen present. A certain amount of calcium was also added whenever superphosphate was applied, but apparently the amount was not sufficient to maintain the exchangeable calcium at the 1931 level.

When the results for the samples from the new area were compared with those for the 1931 samples, considerable changes were also apparent. The loss on ignition and nitrogen results were considerably greater where the soil was in continuous grass, indicating an increase in organic matter content. The amount of exchangeable calcium had increased, whereas the pH value was slightly less than in the 1931 samples though greater than in the 1945 samples from the continuously cultivated area. This increase in exchangeable lime without a corresponding effect on the pH value can be explained by the probable greater exchange capacity due to the presence of larger amounts of organic matter. There was only a slight increase in exchangeable magnesium and none in exchangeable potassium. The readily soluble phosphorus was considerably greater in the sample from the continuous grass area.

Of the crops grown on these plots in 1945, tomatoes and potatoes were common to both areas. The average yields from 24 plots on the old area are compared with those from 24 similarly treated plots on the new area in Table 2. From the results it is readily seen that the productive capacity of the new area for these two crops was twice as great as that of the old area.

From the chemical point of view, the general level of fertility of a soil sample is usually estimated from its reaction and its content of nitrogen, organic matter, exchangeable bases and readily soluble phosphorus. In the results presented above, further evidence has been obtained of the effect of continued cultivation in decreasing soil fertility. It has also been shown that the keeping of this soil in continuous grass for a number of years has resulted in a considerable increase in fertility, measured not only by the chemical factors mentioned but also by crop production when the yields of the old and new areas in 1945 were compared.

It was mentioned earlier in this paper that the soil on these plots closely resembles the Uplands sand. This soil type occurs to a considerable extent in Carleton County (in which Ottawa is situated), amounting to over 13,000 acres, about 2.2 per cent of the total land area of the county (1). It is a soil type of low fertility, and any means for increasing its fertility level should be of importance to all whose farms are located thereon. The results presented in this paper would indicate that periodic retirement of this soil type from cultivation and keeping it in sod would tend to maintain and improve its productive capacity.

SUMMARY

Soil samples from adjacent experimental areas of the Division of Chemistry, Central Experimental Farm, Ottawa, situated on Uplands sand, were taken in 1945. One area has been in continuous grass for 15 years, the other under continuous cultivation. The results of chemical analysis on these two sets of samples were compared with those of soil samples collected from the same location in 1931 when the soil of both areas was considered to be uniform. It was shown that the general level of fertility, as measured by reaction and by content of nitrogen, organic matter, exchangeable bases and readily soluble phosphorus, was decreased by continuous cultivation and increased by keeping the soil in continuous grass. Yields of potatoes and tomatoes in 1945 were much greater on the former grass area, a further indication of the higher level of fertility there. It is suggested that periodic retirement of Uplands sand from cultivation, and keeping it in sod, would tend to maintain and improve its productive capacity.

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SEED GERMINABILITY TESTS WITH 2,3,5-TRIPHENYL TETRAZOLIUM CHLORIDE[1, 2]

R. W. Shuel

Department of Field Husbandry, Ontario Agricultural College, Guelph, Ont.

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The use of 2,3,5-Triphenyl tetrazolium chloride as a test of seed viability was developed by Professor Kuhn, in collaboration with Professor George Lakon of the Bayer Company(1, 2). The test has been used by a large German seed breeding station with oats and rye. Its value lies in the time saved by its use; the test can be completed within a few hours, as compared with the several days required for ordinary germination procedures.

The test is based on the colour change resulting from the reaction of the tetrazolium salt, in aqueous solution, with the tissue of the living embryo of the seed. The colourless tetrazolium compound is reduced to a pink or crimson formazane compound, on addition of a hydrogen radical. This reaction appears to be dependent on respiratory processes in the embryo; if embryos are killed by boiling in water for a few minutes before immersion in the tetrazolium solution, no colour change takes place.

Porter *et al.*(3) conducted a series of tests with tetrazolium at the Iowa Agricultural Experiment Station, using corn, barley, oats, wheat, buckwheat, cotton, peas, rice, soybeans, vetch, and Bahia grass. Per cent germination, as indicated by the tetrazolium salt, agreed closely with that obtained from actual sprouting. In these tests seeds were pretreated by soaking in water for several hours to permit absorption of water into the embryo and the initiation of germination processes. The seeds were then sectioned and immersed in the tetrazolium solution.

The object of the tests described below was to develop a quick method which would require a minimum of equipment. Pretreatment, therefore, was dispensed with.

METHOD

The tetrazolium test was used with several varieties of wheat, oats, and barley. Samples from the same lots were sprouted in moist cloths.

Preliminary tests showed that most of the time taken up in the test was consumed in the penetration of the solution through the hull of the seed. Some samples, therefore, were hulled to determine how much time could be saved.

Seeds to be treated were placed in Petri dishes and covered with a 1% aqueous solution of tetrazolium chloride. Some lots were heated in an attempt to accelerate respiratory activity in the embryo. Results, together with a comparison with sprouted samples, are given in Table 1.

[1] These tests were carried out at the suggestion of Professor R. Keegan, Department of Field Husbandry, Ontario Agricultural College, Guelph, Ont.

[2] The tetrazolium used in the tests was provided by the Research Laboratory of the Dominion Rubber Company, Guelph, Ont.

TABLE I.—SEED VIABILITY AS INDICATED BY GERMINATION TESTS AND TETRAZOLIUM TESTS WITH HULLED KERNELS

Variety	Official germination per cent	Germinated 96 hours		1% Tetra. solution at room temperature				1% Tetra. at 40° C. for 1 hour and 45 min.			1% Tetra. at 40° C. for 2 hours and 40 min.		1% Tetra. at 45° C. for 2 hours		
		Sam. Size	% Germ.	Sam. Size	% Germ. indicated at the end of 3 hr.	6 hr.	22 hr.	Sam. Size	% Germ. indicated at end of 6 hr.	23 hr.	% Germ. indicated at end of 3 hr.	19 hr.	Sam. Size	% Germ. indicated at end of 3 hr.	6 hr.
Montcalm barley*	98.0	200	100.0	200	91.0	97.5	100.0	200	95.5	100	100	—	100	100	—
O.A.C. 21 barley*	98.0	200	99.5	200	92.5	94.5	100.0	200	96.0	99.5	100	—	100	100	—
Beaver oats	96–98.0	200	100.0	200	86.5	98.5	99.0	200	97.5	99	98	100	100	99	—
Beacon oats	—	200	100.0	200	69.5	90.5	95.5	200	96.0	98	96	99	100	100	—
Alaska oats	—	200	100.0	200	83.0	94.0	99.0	200	97.5	99	98	100	100	100	—
Erban oats	99.0	200	97.5	200	93.0	100.0	100.0	200	98.5	100	99	100	100	100	—

* Outstanding malting varieties.

TABLE 3.—RESULTS OF TETRAZOLIUM AND GERMINATION TESTS WITH WHEAT

Variety	Germinated 96 hours		1% Tetra. solution at room temperature			1% Tetra. at 40° C. for 5 hours			1% Tetra. at 45° C. for 5 hours		
	Sample size	% Germ.	Sample size	% Germ. indicated at end of 5 hr.	22 hr.	Sample size	% Germ. indicated at end of 5 hr.	22 hr.	Sample size	% Germ. indicated at end of 5 hr.	22 hr.
Dawson's Golden Chaff	200	96.0	200	88.5	99.0	100	95.0	100.0	200	100	—
Junior No. 6	200	97.0	200	61.5	89.5	100	85.0	98.0	200	99	100
Marquis	200	96.5	200	48.0	70.0	100	74.0	83.0	200	98	98
Coronation	200	88.5	200	94.0	96.0	100	88.0	94.0	200	100	100
Marquis	200	96.0	—	—	—	—	—	—	200	98	98
Coronation	200	98.0	—	—	—	—	—	—	200	100	100

Other samples were immersed in the solution without hulling, and were kept at a temperature of 45° C. to accelerate penetration of the solution through the hulls. Counts were made immediately after removal from the oven. Results are given in Table 2.

TABLE 2.—SEED VIABILITY AS INDICATED BY GERMINATION AND TETRAZOLIUM TESTS WITH UNHULLED SAMPLES

Variety	Germinated 96 hours		1% Tetra. solution at 45° C. for 5 hours	
	Sample size	Per cent germination indicated	Sample size	Per cent germination indicated
Montcalm barley	200	100.0	200	100.0
OAC 21 barley	200	99.5	200	100.0
Alaska oats	200	100.0	200	99.5
Erban oats	200	97.5	200	99.0
Beaver oats	200	100.0	200	97.5
Beacon oats	200	100.0	200	100.0

Some difficulty was experienced at first, particularly with oats, in distinguishing stained embryos through the hulls, but this was overcome by holding the seeds in front of an electric bulb, so that the light diffused through the hull. In this way, even faintly staining embryos could be distinguished.

Samples of four varieties of wheat were treated with a solution of the same strength. Tests were conducted at room temperature, at 40° C., and at 45° C. Results are given in Table 3.

It was felt that samples of seeds with a low percentage germination might provide a more critical test of the efficacy of tetrazolium as a germination indicator. Accordingly, samples of seeds of different ages were tested with the solution; other samples of seeds of the same lots were sprouted for 120 hours in moist cloths. Results of these tests are given in Table 4.

In the case of the old wheat samples, there was a wide variation in the colour of the treated embryos, ranging from carmine to light pink. In many of the lightly coloured seeds, the colouring did not extend throughout the scutellum; such seeds were adjudged not viable. With the oats and barley, coloration was much more uniform, and only seeds which had no colour in the embryo were considered not germinable.

DISCUSSION OF RESULTS

1. Oats and Barley

Hulled Samples.—Close agreement was found between the germinability as indicated by the tetrazolium and by sprouting. Heat appeared to lessen the time required for the reaction to go to completion; at room temperature, nearly 24 hours were necessary; when samples were kept at 45° C. for two hours the reaction required only three hours.

Unhulled Samples.—Here, again, there was close agreement. Heating at 45° C. appeared to accelerate penetration of the solution into the embryo. (At room temperature, with no pretreatment, total time for the test was 24 to 48 hours, with unhulled samples.)

TABLE 4.—COMPARISON OF TETRAZOLIUM WITH SPROUTING TESTS—OLD SEEDS

(100 seed samples)

Variety	% Germination	
	Sprouted—120 hours	Tetrazolium 5 hours at 45° C.
Barley		
1931	81	72
OAC 21 (1945)	99	98
OAC 21 (1935)	88	99
1931	39	45
1935	55	76
Oats		
Victory	93	97
Erban (1941)	91	94
Alaska (1930)	80	83
OAC 144 (1930)	71	75
OAC 72 (1930)	55	52
Alaska (1946)	96	92
Banner (1931)	61	72
Wheat		
Spring (1935)	93	90
Mindum (1941)	68	71
Durum (1945)	64	69
Spring (1935)	81	92
Dawson's (1930)	3	34
Winter (1931)	12	31
Renown (1945)	80	75
Natural Cross (1944)	73	75
Marquis	12	57
Dawson's (1945)	4	23

2. Wheat

There was close agreement between germinated samples and those treated with the solution at 45° C. for 5 hours except in the case of the sample of Coronation wheat. Because of the reddish hue of the seed coat of this variety, identification of stained embryos was difficult. The reaction was much slower in the case of samples treated at lower temperatures, either because of slow penetration into the embryo, or slower respiration.

An attempt was made to establish a relationship between lightly-staining and slow germinating seeds, but a close association was not found.

3. Old Seeds

Considerable variation obtained between the germinability as indicated by the tetrazolium and sprouting tests. The divergence was widest in the case of seeds with a very low percentage germination.

It would appear that sufficient energy is derived from the respiratory processes of these embryos to reduce the tetrazolium, but not enough to promote sprouting. In such cases, the tetrazolium test can be considered as only a very rough index of viability.

SUMMARY

1. Seeds of barley, oats, and wheat were tested for germinability (colour reaction) in a 1% aqueous solution of 2,3,5-tetrazolium chloride. They were kept in covered Petri dishes at a temperature of 45° C. for five hours.

2. This test appears to give a quick, reliable index of germinability with new seeds. For old seeds, whose viability is likely to be less than about 60 per cent, the tetrazolium test is much less accurate.

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PASTURE STUDIES XXVIII. EFFECT OF LIGNIN CONTENT AND OF STAGE OF MATURITY OF DRY CLOVER FORAGE ON THE URINARY EXCRETION OF AROMATIC ACIDS BY SHEEP[1]

J. H. Pazur[2] and W. A. DeLong[3]

Macdonald College, Quebec

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The lignin fraction of forage crops generally is considered to be of low nutritive value to ruminants. Although a digestibility coefficient as high as sixty-four per cent has been reported (2) for this fraction, other estimates recorded in the literature are much lower and range downwards to zero (2, 5, 7, 8, 14). In view of the indefiniteness of present knowledge of the lignin fractions of forage crops and of the empirical nature of the methods available for the estimation of such fractions, the wide variation in the recorded estimates of lignin digestibility is not surprising, especially as a variety of techniques have been employed in obtaining these estimates.

In most of the investigations on lignin digestibility only the fecal excretion of lignin has been measured. Nevertheless, König (12) early suggested that the lignin fraction of the fodder probably furnished the phenyl radical for the formation of the hippuric acid of the urine. Because of the fact that animals consuming high protein diets containing relatively small amounts of lignin normally excrete small amounts of hippuric acid compared to the quantities found in the urine of ruminants, it is commonly believed that the contribution of aromatic amino acids, and of other aromatic substances of non-lignin origin, to the hippuric acid content of the urine of ruminants is small. König's suggestion respecting the origin of this hippuric acid has been supported by experimental evidence. Thus, Csonka *et al.* (7) have reported a marked increase in the excretion of hippuric acid by dogs and by steers when these animals were fed alkali lignin isolated from corn cobs. These authors concluded that a part of the alkali lignin had been metabolized with excretion of the aromatic nuclei as hippuric acid. Sammons and Williams (18) also have shown that vanillin and vanillic acid, possible degradation products of lignin, are excreted in the urine by the rabbit. Further, Brigl and Pfähler (4) found that rabbits and sheep excreted about three times as much benzoic acid when fed on hay as when fed on straw. They demonstrated that the major part (58 per cent) of the increased benzoic acid excretion had its origin in the fraction extracted from the hay by alcohol containing 2 per cent HCl. It was also found that pre-treatment of the hay with alkaline reagents reduced the production of hippuric acid.

A number of the investigators of the lignin problem hold the view that lignin is a complex substance which is modified in composition and in complexity as the cells or tissues containing it mature. Thus, Freudenberg (9) has expressed the opinion that lignin exists in wood in different degrees of condensation ranging from simple units of rather low molecular weight to

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[1] Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Quebec. Journal Series No. 235.

[2] Graduate Assistant to the Macdonald College Pasture Committee.

[3] Associate Professor of Chemistry.

highly complex aggregates. The existence of a soluble lignin fraction of comparatively low molecular weight in woody tissues is supported by the experiments of Brauns (3). Phillips *et al.* (15, 16) also have shown that both the apparent lignin content and the methoxyl content of the lignin fractions isolated from developing oat and barley plants increase with increasing maturity of the tissues.

Since relatively few investigations of the variation in the aromatic acid content of the urine of herbivora have appeared in the literature, and since the available evidence indicates that the lignin fraction of plant tissues changes in amount and nature with the maturity of these tissues, it seemed desirable to study the effect of the lignin content and of the stage of maturity of a forage crop on the urinary excretion of aromatic acids by a ruminant. This paper reports the results of such a study of the urine of sheep fed red clover hay of varying degrees of maturity.

EXPERIMENTAL MATERIALS AND METHODS

The forage used in these experiments consisted of red clover plants harvested at five stages of growth in each of the seasons of 1944 and 1945. The dates of harvesting and the anatomical composition of the clover samples are given in Table I. The data for this table were obtained by analysing the freshly cut forage. In Tables 3 and 5 below sample numbers refer to cuttings of differing maturity as indicated in Table 1.

TABLE 1.—ANATOMICAL COMPOSITION*

Sample Number	Date of Cutting	Leaf Laminae	Stem and Stipules	Stem-Stipule / Leaf Laminae Ratio	Bloom
		%	%	%	%
1	May 26, 1944	46.4	53.6	1.16	0.0
	June 1, 1945	39.3	60.7	1.55	0.0
2	June 1, 1944	33.8	63.2	1.87	3.1
	June 8, 1945	34.9	64.1	1.84	1.0
3	June 12, 1944	24.4	63.1	2.59	12.4
	June 21, 1945	25.6	69.8	2.73	4.6
4	June 22, 1944	22.0	61.6	2.80	16.5
	June 30, 1945	19.1	71.6	3.75	9.3
5	July 10, 1944	18.4	56.1	3.05	25.5
	July 12, 1945	15.9	71.6	4.50	12.5

* Data supplied by W. J. Drapala and W. C. Proctor, graduate assistants to the Macdonald College Pasture Committee.

In 1944 the forage was dried in a Wisconsin-type artificial corn-drier consisting of six compartments each 5′ × 5′ × 7′. A current of air at a temperature of 43° to 45° C. was circulated through each compartment. The clover was placed in this apparatus as soon as possible after cutting. Under the conditions of operation approximately constant weight was attained in two to three days. The dried material was stored for the feeding trials, which began eight months later. These forage samples were fed in *Experiment I.*

In 1944 samples for analysis were selected from the fresh material soon after cutting. Each of these sub-samples was dried in a sack in the drier along with the bulk samples which were to be used in the feeding experiments. When dry, the analytical sub-samples were cut with scissors into pieces one to two inches in length, then ground in a Wiley mill until the whole sample passed a 0.5 mm sieve.

In 1945 the forage was air-dried on tripods in the field. Precipitation during this season was higher than usual. Because of weather conditions and because of somewhat more handling in the field, the forage samples collected in 1945 might be expected to be somewhat different from those obtained in 1944. These factors may, to some degree, account for the higher stem to leaf ratio of the 1945 samples (cf. Table I). When dry the 1945 forage was chopped in a silage cutter and stored for the feeding tests of *Experiment 2*. The length of this storage period was about two months. Representative samples for analysis were selected from the chopped material at the time of feeding. These samples were ground prior to analysis as in 1944.

The experimental animals were pure-bred Cheviot wethers aged about sixteen months. Three sheep were used for the experiments. Each sample was fed to one sheep during the feeding trials.

All forage samples were chopped before feeding. This was to ensure, as far as possible, complete consumption of the material fed with minimum rejection of stem tissues. The daily ration of each forage sample was 800 grams per sheep. Five days' preliminary feeding of each sample preceded the actual feeding tests.

In *Experiment 1* the animals were on test for 48 hours. During this time the urine voided was collected every twelve hours, measured, and aliquots of one-tenth the total volume withdrawn and preserved by saturation with benzoic acid. The hippuric acid content of these urine samples was determined by the method of Griffith (10). In *Experiment 2*, the duration of the test was 24 hours. The urine samples analysed consisted of the total urine voided during this period. No urine preservative was used. There was no visible evidence of decomposition of the urine during this interval. These samples were analysed immediately after collection for their hippuric acid and total benzoic acid contents. The method employed for the determination of total benzoic acid was that of Kingsbury and Swanson (11).

In order to obtain evidence respecting the validity of the assumption that hippuric acid remained constant in sheep urine during the 24-hour period following the voiding of the urine, the following experiment was conducted. Sheep urine was analysed immediately after excretion and at 24-hour intervals for a 72-hour period. The results of this experiment are shown in Table 2.

Inspection of the data shows that the hippuric acid content remained constant for at least 24 hours, and that the loss of this acid was low up to 48 hours. Visual observations made during the course of the investigation, as well as the results of this experiment, indicated that losses in hippuric acid content generally were accompanied by the formation of a fine yellowish-brown sediment in the urine.

TABLE 2.—RATE OF DECOMPOSITION OF HIPPURIC ACID IN SHEEP URINE

Time, Hours	Hippuric Acid/5 ml. of Urine, Mgs.	Loss of Hippuric Acid, Per Cent
0	99.3 ± 0.9 (2)*	
24	100.0 ± 0.5 (2)	−0.7
48	90.4 ± 0.4 (2)	9.0
72	61.6 ± 0.0 (2)	38.0

* Figures in brackets refer to the number of determinations.

TABLE 3.—PROXIMATE COMPOSITION OF 1944 SAMPLES

Sample No.	Dry Matter	Crude Ash	Crude Protein	Crude Fat	Soluble Carbo-hydrates	Cellu-lose	Lignin		1945 Samples Lignin Det'd
							By Diff.	Det'd	
	%	%	%	%	%	%	%	%	%
1	90.5	9.0	27.2	4.1	38.3	13.8	7.6	7.3	7.6
2	90.6	8.0	22.6	3.3	40.7	17.4	8.0	7.7	10.1
3	91.4	6.7	17.5	2.8	44.9	18.7	9.4	7.9	11.9
4	90.8	6.7	16.2	2.6	43.0	20.8	10.7	9.7	12.9
5	89.3	6.2	14.8	2.7	40.2	21.5	14.6	12.2	15.2

EXPERIMENTAL RESULTS

The analytical data for the 1944 hay samples used in *Experiment 1* are shown in Table 3. This table also contains data on the apparent lignin contents of the forage samples collected in 1945 and fed in *Experiment 2*. In the determination of dry matter, crude protein, crude ash and crude fat, the methods of estimation were essentially those of the A.O.A.C. The soluble carbohydrate, cellulose, and lignin by difference estimates were obtained by the procedures outlined by Crampton and Whiting (6). The direct determination of lignin followed the procedure of Manning and DeLong (13), Table 3.

It will be noted that the estimates of the lignin contents of the 1944 samples obtained by the two methods employed are in reasonably good agreement. Table 3 also shows that the apparent lignin contents of the 1945 samples are, with the exception of that of the first cutting, considerably higher than those obtained by the same method for the 1944 samples. Reference to the data on stem stipule/leaf laminae ratios given in Table 1 shows that these ratios are not well correlated with the differences in apparent lignin content shown in Table 3. Neither, if one may judge from the percentage of bloom shown in Table 1, are these differences in apparent lignin content attributable to differences in physiological maturity at the times of cutting in the two years. It is possible that they may be related to the manner of curing the samples.

Since urine is subject to bacterial decomposition resulting in destruction of hippuric acid, it was essential to establish the conditions of storage necessary for the preservation of the latter substance. Experience showed that saturation with toluene or addition of a small amount (0.15 per cent)

of benzoic acid were ineffective. It was therefore decided to determine whether or not saturation of the urine with benzoic acid would have the desired effect. To this end a solution containing 3.00 gms. of hippuric acid per liter was prepared. Analysis of 5 ml. aliquots of this solution by the method of Griffith (10) gave recovery of the expected amount of the acid. An aliquot of this hippuric acid solution then was saturated with benzoic acid, allowed to stand at room temperature for 10 days, and re-analysed. Satisfactory recovery of hippuric acid was obtained both in the case of this standard solution and also with samples of urine similarly preserved. Accordingly, this method of preservation was followed in *Experiment 1.* Some of the experimental data obtained in the investigation of the conditions required for the preservation of urine samples for the determination of hippuric acid contents are given in Table 4.

TABLE 4

Material	Preservative	Mgms. of Hippuric Acid/5 ml.		Recovery, %
		First Day	Tenth Day	
Standard solution	None	15.0	14.6	97.3
	Saturated with benzoic acid	15.1	15.0	99.3
Sheep urine No. 1	None	61.0	5.8	9.5
No. 1	Saturated with toluene	62.3	3.6	5.8
Sheep urine No. 2	0.15% benzoic acid added	55.2	2.1	3.8
Sheep urine No. 3	Saturated with benzoic acid	48.2	47.5	98.6

The results of the analysis of the urine samples obtained in the two feeding experiments are shown in Table 5. In addition to the excretion of hippuric acid (*Experiment 1*) or of hippuric acid and total benzoic acid (*Experiment 2*), this table also gives the quantity of lignin consumed during the test period, and the excretion of aromatic acids calculated to the uniform basis of one hundred grams of lignin intake. The lignin consumption is calculated from the value for apparent lignin content obtained by direct determination using 72 per cent sulphuric acid (13). The term "Other Benzoic Acid", used in connection with *Experiment 2*, refers to the difference between the total amount of benzoic acid excreted and the amount excreted in the form of hippuric acid. In this connection it must be emphasized that, in reporting these results, it has been assumed that all the acidic material isolated from the urine by the method of Kingsbury and Swanson (11) is benzoic acid, and similarly, that the acid content of the extract obtained by the procedure of Griffith (10) consists solely of hippuric acid. In neither instance is the presence of other acidic substances of similar solubility relations excluded.

DISCUSSION

Reports in the literature, (4, 7, 12) although few in number, agree that the relatively high concentration of hippuric acid in the urine of herbivorous animals originates, mainly at least, in the lignin fraction of the plant

TABLE 5

Experiment 1 (1944): Duration of Test Period 48 hours

Sample No.	Lignin Fed, Gms.	Total Hippuric Acid Excreted during Test-Period Gms.	Hippuric Acid Excreted per 100 Gms. Lignin Fed Gms.
1	106	39.5	37.3
2	112	31.4	28.1
3	116	24.6	21.3
4	141	21.3	15.1
5	174	20.1	11.5

Experiment 2 (1945): Duration of Test-Period 24 hours

Sample No.	Grams Lignin Fed	Excretion of Acids per 100 gms. Lignin Fed			Other Benzoic Acid
		Hippuric Acid	Total Benzoic Acid	Benzoic Acid Excreted as Hippuric Acid	
		Gms.	Gms.	Gms.	Gms.
1	54	6.52	6.98	4.40	2.58
2	71	4.08	5.57	2.77	2.80
3	85	3.78	3.20	2.57	0.63
4	92	3.11	2.76	2.12	0.64
5	110	2.34	2.12	1.61	0.51

tissues constituting their food. The amount of hippuric acid formed from phenolic amino acids and other aromatic compounds of non-lignin origin is deemed to be relatively small. Assuming these conclusions of previous workers to be correct, the relative excretion of hippuric and benzoic acid obtained in the present experiments indicates that the lignin fraction of hay from immature clover plants finds its way more readily into the urinary tract of the sheep than does that of hay from more mature clover. Thus, the data of Table 5 show that about three times as much hippuric acid and total benzoic acid were excreted (per 100 grams of apparent lignin consumed) from the earliest cut hays as from the most mature hays. In the case of hippuric acid the relative excretion from the youngest as compared to the oldest hays of a given season was closely similar in the two experiments, notwithstanding the far higher excretion in *Experiment 1.*

The data of Table 5 also show that relatively large quantities of "Other Benzoic Acid" may be excreted, particularly when hay from young clover plants is fed. It is thus apparent that the total benzoic acid excreted cannot be taken as a measure of the elimination of hippuric acid, as has sometimes been done (4, 11). It may be pointed out here that our review of the literature indicates that relatively little is known of the nature of the aromatic compounds which may be present in the urine of ruminants.

Bacterial decomposition in the rumen may play a significant role in the metabolism of the lignin fraction of plant tissues by herbivora. Although apparently relatively resistant to attack by micro-organisms the lignin

fraction has been found to be susceptible, under certain conditions at least (1, 17, 19). It is, therefore, quite possible that the apparently greater metabolism of the lignin of immature plant tissues by sheep in the present experiments may have been brought about through attack by micro-organisms. Experiments by Waksman (19) indicated that the lignin fraction of young rye plants was about three times as susceptible to such attack as was that of mature rye plants. It is suggested that the greater hippuric acid excretion obtained from the feeding of the 1944 hays possibly may have been due to the presence of a more active (relative to lignin) micro-flora in the rumen of the sheep used in *Experiment 1.*

SUMMARY

(1) Clover forage cut at five stages of growth was fed to sheep and the relative amounts of aromatic acids excreted in the urine were determined.

(2) It was found that sheep fed clover harvested at an early stage of growth excreted about three times as much hippuric acid and benzoic acid as did sheep fed clover harvested at the most mature stage of growth.

(3) The data suggest that lignin units present in the earlier stages of growth of the clover plant are more readily metabolized by ruminants than those present in more mature clover.

(4) It is pointed out that attack upon the lignin fraction may be dependent on the activity of the micro-flora of the rumen.

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THE CONTROL OF *PENICILLIUM* SPP. ON TULIP AND IRIS BULBS IN STORAGE BY FUNGICIDAL DUSTS[1]

By J. E. Bosher[2] and W. Newton[3]

Dominion Laboratory of Plant Pathology, Saanichton, B.C.

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During the interval between harvesting and replanting a great amount of injury is caused by *Penicillium* spp. on tulip and iris bulbs. The injury is negligible when the bulbs are maintained in a dry atmosphere under well ventilated conditions, but after prolonged periods of wet or humid weather the losses are frequently high. The tulip and iris bulb growers of British Columbia have attempted to lower these losses by dusting the bulbs in the storage racks with sulphur. As judged by the amount of mould on bulbs so treated, this practice has little value. A comparison was made, therefore, of the effectiveness of a number of fungicidal dusts, including sulphur, in the control of the mould.

METHOD

Two hundred tulip bulbs (variety *Orange King*) and the same number of iris bulbs (variety *Wedgewood*) were divided into lots of ten, the tunics were removed, and with the exception of the controls, each lot was dusted by shaking it in a paper sack containing an excess of fungicide. Prior to the treatment with the fungicidal dusts, one-half the total were injured by scraping the bulb surfaces with a knife. After the application of the fungicidal dusts, all the bulbs were placed on the surface of moist peat and loosely covered with oiled paper. At regular intervals a record was made of the number of bulbs of each lot that developed visible evidence of mould. At the end of the humid storage period, the area covered by mould on each bulb was estimated quantitatively, and then expressed by numerical values from 0 to 10. The mean of the estimates for each lot is recorded in Table 1 as the degree of mould development. These estimates of the areas covered with mould on the surfaces of the injured bulbs were made 21 days after the bulbs were placed in moist storage, because at that time a large number of the unprotected bulbs had become completely covered with mould. On the other hand, the estimates of the areas covered with mould on the untreated bulbs were made 36 days after the bulbs were placed in moist storage. Only after 36 days of this treatment had sufficient mould developed to make accurate estimates of the comparative degree of mould development as influenced by the fungicidal dusts.

DISCUSSION

Spergon, Arasan, Dow No. 5, and Nomersan were effective in preventing the development of *Penicillium* spp. on both injured and normal tulip and iris bulbs maintained under humid storage conditions. The ratings for mould coverage on the treated bulb surfaces ranged from 0.0 to 1.5, and from 6.0 to 8.5 on the untreated. On bulbs treated with Fermate, Dithane, two forms of sulphur dust, and a proprietary Bordeaux, the ratings ranged from 4.5 to 9.9, thereby indicating that these dusts exerted no practical degree of protection.

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[1] Contribution No. 915 from the Division of Botany and Plant Pathology, Dominion Department of Agriculture, Ottawa, Canada.

[2] Technician, *Gr. 2*, Dominion Laboratory of Plant Pathology, Saanichton, B.C.

[3] Officer-in-Charge, Dominion Laboratory of Plant Pathology, Saanichton, B.C.

TABLE 1.—THE DEGREE OF MOULD DEVELOPMENT ON THE SURFACES OF TULIP AND IRIS BULBS UNDER MOIST STORAGE AS INFLUENCED BY FUNGICIDAL DUST TREATMENTS

Treatment	Period of storage			
	21 days	36 days	21 days	36 days
	Injured tulips	Normal tulips	Injured iris	Normal iris
Spergon	0.5	0.0	0.5	0.5
Arasan	1.5	0.5	0.5	0.5
Nomersan	1.0	1.5	1.0	1.5
Dow No. 5	0.0	0.0	0.5	0.5
Fermate	7.5	5.0	6.5	4.5
Dithane*	9.0	7.5	7.5	8.0
Sulphur	7.0	6.0	6.0	6.5
Kopper's sulphur	6.5	5.5	5.0	5.5
Bordeaux	5.0	5.5	—	—
Untreated	8.5	6.5	8.0	6.0

* *Rhizopus* spp. in addition to *Penicillium* spp. developed upon the bulbs dusted with Dithane.

When lots of the same bulbs were immersed for one hour in 1 per cent solutions of wettable Spergon, wettable sulphur, Fermate, and Dithane, after a 21-day moist storage period, the mould coverage ratings were 9.0, 9.0, 10.0, and 7.0, respectively. The heavy mould growth on the bulbs indicated that the re-wetting of bulbs with fungicidal solutions prior to storage was not a beneficial practice. Of passing interest is the fact that a wettable form of Spergon was slightly less effective than the normal form when both were applied as dry dust treatments. With the former the mould coverage rating was 1.5 and with the latter 0.5. The hygroscopic nature of the wettable form probably allows a more rapid absorption of water by the bulb tissue and the growth of *Penicillium* spp. is undoubtedly affected by the moisture content of the bulb tissue.

SUMMARY

Spergon, Arasan, Dow No. 5, and Nomersan dusts effectively prevented the development of *Penicillium* species on both injured and normal tulip and iris bulbs maintained under moist storage conditions. Fermate, Dithane, two forms of sulphur, and a proprietary Bordeaux dust exerted little or no protective effect.

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